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STUDIES ON THE ULTRAFILTRABILITY OF SERUM SODIUM AND POTASSIUM

BY

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PREFACE

This work was carried out in the Wihuri Research Institute, Helsinki, in 1958—1960. It is a pleasure to express my gratitude to the head of the Institute, Professor Pentti I. Halonen, M.D., also head of the First Medical Clinic, University of Helsinki, who supervised my work through all its stages, giving me the benefit of his wide knowledge and constructive criticism and placing the excellent laboratory facilities of the Institute at my disposal.

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Helsinki, March 1961

S. S.

CONTENTS

REVIEW OF THE LITERATURE ON THE DIFFUSIBILITY OF SERUM SODIUM AND POTASSIUM ACROSS A SEMIPERMEABLE MEMBRANE

The state of alkali metal ions in the serum is not a new subject of investigation. Even in 1850 Schmidt, in Estonia, discussed in detail the amount of sodium bound to serum proteins and the significance of this binding in the equilibrium of sodium with intestinal contents and in the loss of sodium in cholera diarrhea. The literature concerning alkali metals in biology and medicine is now voluminous, but its study is aided by some excellent reviews. Manery (1954) and Thorn (1960) have published fairly recent surveys of studies of the state of sodium and potassium in various body fluids. The methods for study of the interactions of alkali metals with macromolecules have been reviewed by Klotz (1953).

One of the most common methods for study of the state of small ions and molecules in fluids is the utilization of the properties of a semipermeable membrane allowing the passage of only small particles. This may be done either by the equilibrium dialysis method, by which the concentration of small particles diffusing across a membrane into the dialysis fluid is determined, or by the ultrafiltration method, by which the fluid to be investigated is forced across a membrane by means of mechanical pressure. The composition of the fluids obtained by equilibrium dialysis and ultrafiltration is not different thermodynamically (Ingraham *et al.* 1933). The following review is limited to studies by these two methods of the state of sodium and potassium in serum or plasma.

The nomenclature introduced by Brönsted (1923) has been used throughout this work in describing the electrolyte and acid-base balance. The theoretic and practical advantages of this terminology are emphasized by recent Danish authors (e.g., Astrup 1954, Prætorius

1954, Møller 1959). According to Brönsted, acids are referred to as proton donors and bases as proton acceptors. Consequently, for instance alkali metal ions are not bases, as they sometimes have been regarded in clinical chemistry.

CERTAIN EARLY SERUM ULTRAFILTRATION AND DIALYSIS EXPERIMENTS

In the latter half of the 19th century the composition of the fluid filtrated from serum through a semipermeable membrane became a subject of interest to physiologists. One of the reasons was the theory expressed by Ludwig in 1844 that the glomerular filtrate is an ultrafiltrate forced mechanically from plasma through the glomerular membrane. The theory of diffusion of substances across a porous membrane, based on thermodynamics, was presented by Fick (1855). Some decades later Martin (1896) observed that when serum is forced across a gelatin membrane albumin and globulin remain behind the membrane but the "crystalloids" pass through in the same ratio as water. Starling (1899), using a gelatin membrane, found that the freezing points of serum and its ultrafiltrate are nearly the same, which points to the probability that serum inorganic salts are almost completely ultrafiltrable. Reid (1901), on the other hand, reported a higher freezing poit of ultrafiltrate than of serum. However, the ultrafiltration pressure used by him was very high, 30 — 55 atm., and it was shown by Burian (1909) that the use of such high pressures raises the freezing point of the ultrafiltrate.

Loewy and Zunz (1894) found that titrable alkalinity is dialyzed through a parchment membrane at a much slower rate from serum than from soda solution, and that a part of the serum "alkali" is therefore in nondiffusible form. Rona (1910) reported that the chloride content of the dialysis fluid of serum is greater than that of serum. He ascribed this difference to the serum proteins, which would form their own volume so that the concentration of substances present in serum water, such as chloride ion, is greater per volume of serum water than per volume of whole serum. In the following year Donnan demonstrated that proteins may influence distribution of ions for also another reason.

DONNAN'S THEORY

Based on the thermodynamic laws of Gibbs, published in English in 1906, Donnan developed in 1911 his theory of the manner in which dissociated colloids influence the distribution of small ions across a semipermeable membrane, as follows: On the one side of the membrane there is a dissociated protein salt Na^+ $prot^-$ and on the other side salt Na^+Cl^-. The protein anion cannot pass through the pores of the membrane, whereas Na^+ and Cl^- are capable of doing so.

Na^+ $prot^-$	Na^+Cl^-
$y+z$ z	x x
Cl^-	
y	

Letters y, z and x denote the concentrations of the ions at equilibrium. It is assumed that the conditions are such that the ionic activity coefficients are equal on both sides. Then, in equilibrium according to Donnan:

(equation 1) $$y(y + z) = x^2.$$

When equation 1 is applied to serum, then $(y + z)$ = serum sodium concentration per liter of serum water = $(Na)_s$; x = ultrafiltrate sodium concentration per liter of ultrafiltrate water = $(Na)_f$: z = cation equivalency of serum proteins* per liter of serum water = $(prot^-)_s$; y = serum diffusible anion concentration per liter of serum water = $(Na)_s - (prot^-)_s$. When these values are placed into equation 1 and when $(prot^-)_s$ is solved, we obtain

(equation 2) $$(prot^-)_s = (Na)_s - \frac{(Na)^2_f}{(Na)_s}.$$

When the sodium concentration in serum water and the cation equivalency of serum proteins are known, the sodium concentration in the ultrafiltrate water may be calculated as follows:

(equation 3) $$(Na)_f = \sqrt{((Na)_s - (prot^-)_s)\,(Na)_s}.$$

*) Cation equivalency of serum proteins = number of equivalents of cations matching the excess of negative charges of the plasma proteins (Thorn 1960).

For practical purposes it can be calculated with sufficient accuracy from equation 3 that the difference in the sodium concentrations in serum and ultrafiltrate water is according to Donnan's theory one-half of the cation equivalency of serum proteins. Thus, for example, if the cation equivalency of serum proteins decreases from 20 mEq./l. H_2O to 10 mEq./l. H_2O, the difference in the sodium concentrations of serum and ultrafiltrate is reduced from 10 mEq./1. H_2O to 5 mEq./l. H_2O. In the applications given above, the total cation concentrations of serum and ultrafiltrate should be used instead of the sodium concentrations of these fluids. In practical application this circumstance is of fairly small significance, since about 92 per cent of the serum total cations consist of sodium.

Using the equation of Nernst (1889), Donnan also developed the equation for membrane potential, i.e., for the potential difference of solutions on the two sides of the membrane, as follows:

(equation 4) $$E = \frac{RT}{F} \ln \frac{c1}{c2}.$$

where c1 and c2 indicate the activity of the same ion in the two phases. When the potential difference is expressed in millivolts and the temperature is 38° C, the formula for membrane potential may be written

(equation 5) $$E_{mV} = 62 \log \frac{c1}{c2}.$$

The origin of the Donnan membrane potential is the diffusion potential arising from the low mobility of protein ions. The same potential difference is measurable also in the absence of a membrane when the protein phase is being diffused in the liquid (Hitchcock 1955).

EFFECT OF DISSOCIATION OF SERUM PROTEINS ON DISTRIBUTION OF IONS

In order to calculate the effect according to Donnan's theory of serum proteins on the distribution of ions, we must know how the proteins are dissociated. This subject was studied by Van Slyke *et al.* (1928) using a special carbon dioxide titration method. They found

that at physiologic pH the serum proteins are dissociated into anions according to the following equation:

(equation 6)
BP_S (= 0.78 (alb. N) (pH − 5.16) + 0.48 (glob.N) (pH − 4.89)

in which BP_S = "base bound by serum proteins". If this term is substituted by the modern term "cation equivalency of serum proteins" (Manery 1954), and the protein concentration stated per gram of protein nitrogen is converted to the concentration per gram of protein by using the factor 6.25, the above equation will be changed to

(equation 7) $prot^-_S$ mEq./l. = 1.25 (gm.alb/100 ml.) (pH − 5.16) + 0.77 (gm.glob/100 ml.) (pH − 4.89).

Assuming that, for example, the serum albumin concentration is 4 gm. per 100 ml., the globulin concentration 3 gm. per 100 ml., pH 7.40, sodium concentration 140 mEq./l., and water content 94 gm. per 100 ml., calculation by equations (7) and (3) will give 140/149 = 0.94 as the ratio of the sodium concentrations in the ultrafiltrate water and the serum water. This ratio is the so-called theoretic Donnan ratio, or the Donnan factor. Provided the serum does not contain substances that selectively change the thermodynamic activity of some ion, the ultrafiltrability ratio of all the diffusible cations should be equal, and the ultrafiltrability ratio of the diffusible anions should be the inverse.

EXPERIMENTS ON DIFFUSIBILITY OF SERUM SODIUM AND POTASSIUM

Table 1 is a compilation of experimental results collected from the literature on the diffusibility of sodium and potassium of normal serum. As is seen, the percentage of ultrafiltrable sodium corresponds fairly well to the theoretic Donnan ratio. The percentage of ultrafiltrable potassium varies greatly in the experiments of different investigators. In the early reports, in which the determinations of sodium and potassium were made by chemical methods, the percentage of ultrafiltrable potassium generally was lower than that of sodium (e.g., Greene and Power 1931, Sjollema and Seekless 1933, Ingraham *et al.* 1933), whereas in the more recent studies

using flame photometry (Berliner *et al.* 1950, Tarail *et al.* 1952) or radioactive isotopes (Dawson 1955) the percentages of ultrafiltrable sodium and potassium were approximately equal. According to Tarail *et al.* (1952), the low ultrafiltrability of serum potassium in older experiments is apparently due to methodical error.

Table 1.— *Diffusibility of Sodium and Potassium from Normal Serum*

Author	Year	Subject	No. of Experiments	Method	$\frac{100(Na)_f}{(Na)_s}$ * %	$\frac{100(K)_f}{(K)_s}$ * %
Rona and Göyrgy	1913	Horse	8	Dialysis	100	—
Richter-Quittner	1921	Human	7	Ultrafiltration	100**	60**
Neuhausen and Pincus	1923	Pig	3	Ultrafiltration	100**	100**
Rona and Petow	1923	Not stated	8	Dialysis (pH 7.0—8.0)	100**	100**
Richter-Quittner	1924	Human	4	Ultrafiltration	—	100**
Tschimber and Tschimber	1924	Human	2	Ultrafiltration	100**	—
Hastings *et al.*	1926	Horse	3	Dialysis	90.6	82.1
Brull	1930	Dog	2	Ultrafiltration *in vivo*	90***	—
Greene and Power	1931	Dog	15	Dialysis *in vivo*	90.8***	78.0***
Scholtz	1931	Bovine	3	Ultrafiltration	—	82 7
Scholtz	1932	Human	2	Ultrafiltration	—	100
Ingraham *et al.*	1933	Dog	17	Ultrafiltration	92.6	85.6
Sjollema and Seekless	1933	Rabbit	12	Ultrafiltration	97.0**	78.5**
Rotschild	1939	Dog Cat	3 2	Ultrafiltration	—	83.7**
Somogyi	1940	Dog Cat	6 1	Dialysis	—	86.8**
Schönholzer	1942	Human	2	Dialysis	—	88.3
Berliner *et al.*	1950	Human Dog	2 3	Ultrafiltration	92.0*** 92.0***	90.0*** 92.0***
Tarail *et al.*	1952	Human	16	Ultrafiltration	94.2	94.3
Dawson	1955	Rabbit	> 5	Dialysis	94.5***	96.0***

* Percentages of diffusible serum sodium and potassium are calculated from the sodium and potassium concentrations of serum water and ultrafiltrate or dialysate water stated by the authors.
** Sodium and potassium concentrations were stated per liter of whole fluid, not per liter of water.
*** Experiment carried out with plasma.

Loeb *et al.* (1922) were probably the first to study whether the distribution of ions in plasma and edema fluid corresponds to the distribution of ions across an artificial membrane. They determined the potassium and chloride concentrations of serum and edema fluid, following which these fluids were dialyzed against each other across a collodium membrane. There was no change in the concentration of these ions after dialysis. It would therefore appear that the distribution of the studied ions between the plasma and the edema fluid

depends on a simple membrane equilibrium and is not entirely due to properties peculiar to living protoplasm. Most investigators (Gollwitzer-Meir 1925, Hastings *et al.* 1926, Greene *et al.* 1931, Gilligan *et al.* 1934) have been of the opinion that the plasma sodium and potassium are distributed in the ascites and edema fluids in accordance with Donnan's theory.

Using venous stasis, Gerbrandy *et al.* (1960) forced ultrafiltrate into the interstitial space of normal human subjects and calculated the amount of protein-bound sodium and potassium on the basis of the sodium, potassium and protein concentrations of plasma samples taken before and immediately after the stasis. According to their results, about 9 per cent of the sodium and 21 per cent of the potassium was bound to the serum proteins, which is in good agreement with the results of ultrafiltration across artifical membranes shown in table 1.

It therefore seems probable from the literature that sodium and potassium are distributed from the plasma into ascites and edema fluids and into fluid forced by venous statis into the interstitial space, in the same manner as they are distributed into an ultrafiltrate forced across a nonliving membrane. It may be assumed, howerer, that when the interstitial fluid contains polyelectrolytes that bind sodium and potassium, the composition of the interstitial fluid differs from that of the plasma ultrafiltrate (Thorn 1960). It is known that, for instance, the colloidal matrix of the connective tissue binds sodium and potassium (e.g., Kulonen 1953, Joseph *et al.* 1954, Farber 1960). It is also known that the sodium and potassium concentrations of cerebrospinal fluid (Flexner 1934, Cooper *et al.* 1955) and aqueous humor (Dawson 1955) differ, although slightly, from the concentration of fluids obtained *in vitro* by ultrafiltration or dialysis of plasma.

DIFFUSIBILITY OF CERTAIN OTHER SERUM IONS

In the 1920's and 1930's, when Donnan's theory became more widely known and investigation of the diffusibility of serum ions was most active, the determination of sodium and potassium was laborious with the available chemical methods. These ions therefore were given much less attention than certain other serum ions, such as chloride and calcium. Rona (1910) found that the chloride concentration of

the dialysis fluid of serum is higher than that of the serum. After publication of Donnan's theory a number of investigators (e.g., Loeb *et al.* 1922, Gollwitzer-Meier 1925, Hastings *et al.* 1926, Greene *et al.* 1931, Muntwyler *et al.* 1931, Gilligan *et al.* 1934) have uniformly reported the finding that the chloride ion is distributed in conformity with Donnan's theory from serum or plasma into transsudation fluids formed in the body, as well as into dialysis or ultrafiltration fluids forced across an artificial membrane. Gilligan *et al.* (1934), furthermore, observed in a heterogeneous series of patients that in accordance with Donnan's theory the difference between the chloride concentrations of transsudation fluids and serum increases with increasing difference in the protein concentrations.

Concerning serum calcium, Rona and Takahashi (1911) were the first to observe that only 65—75 per cent of it is dialyzable. Since then, the dialyzability and ultrafiltrability of calcium has been studied intensively, as is seen from the survey published by, e.g., Prasad (1960). The diffusibility ratio of calcium is considerably smaller than the theoretic Donnan ratio if the diffusibility ratio is calculated on the basis of the serum total calcium. When it is calculated from the "ionized" calcium, the amount of which is obtained by using the pK_{CaProt} value of 2.22 presented by McLean and Hastings (1935), the diffusibility ratio of calcium is actually higher than it is when calculated according to Donnan's theory (Manery 1954). The reason may be that a part of the calcium is ultrafiltrated as small-molecular complexes whose formation increases the diffusibility of calcium (Manery 1954). The ultrafiltrability of calcium depends chiefly on the serum proteins, mostly on albumin: i.e., the lower the albumin concentration, the higher the percentage of diffusible calcium (e.g., Hopkins *et al.* 1953, Prasad and Flink 1958). The percentage of ultrafiltrable magnesium is likewise dependent chiefly on the serum albumin content (Copeland and Sunderman 1952, Prasad *et al.* 1959).

The hydrogen ion is distributed from the serum across a collodium membrane into the dialysis fluid in the same ratio as it is distributed in the body between the plasma and the edema or ascites fluid, but the ratio of distribution is slightly lower than Donnan's theoretic ratio (Hastings *et al.* 1926). In the opinion of Gollwitzer-Meier (1925) the distribution of the hydrogen ion between plasma and edema or ascites fluid may be explained on the base of Donnan's theory.

DIFFUSIBILITY OF SERUM SODIUM AND POTASSIUM IN PATHOLOGIC CONDITIONS

It is seen from table 2 that the distribution of serum potassium across an artificial membrane in pathologic conditions has been studied more than the distribution of sodium. Scholz (1932) found that especially hyperkalemic patients with renal insufficiency have a reduced percentage of ultrafiltrable potassium. Schönholzer (1942), on the other hand, found an increased percentage of dialyzable potassium in this disease. Somogyi (1940) reported an increase in the percentage of dialyzable serum potassium following adrenalectomy. Jantz (1947) observed in patients with familial periodic paralysis that when the potassium concentration of serum is lowest during an attack, its concentration in the ultrafiltrate is relatively still lower. Tarail *et al.* (1952) determined the percentage of ultrafiltrable sodium and potassium in the serums of a group of healthy persons and a group of patients, the latter consisting chiefly of

Table 2. — *Diffusibility of Sodium and Potassium from Pathologic Serum*

Author	Year	Method	Diagnosis	No. of Experiments	$\frac{100(Na)_f}{(Na)_s}$ % *	$\frac{100(K)_f}{(K)_s}$ % *
Scholtz	1932	Ultrafiltration	Renal insufficiency	26	—	87.0
			Controls	2	—	100
Rotschild	1939	Ultrafiltration	Post-adrenalectomy condition	5	—	91.1**
			Controls	5	—	83.7**
Somogyi	1940	Dialysis	Post-adrenalectomy condition	8	—	95.0**
			Controls	7	—	86.8**
Schönholzer	1942	Dialysis	Renal insufficiency	6	—	90.4
			Controls	2	—	88.3
Jantz	1947	Ultrafiltration	Familial periodic paralysis	1	—	60.0**
			Control	1	—	100 **
Tarail *et al.*	1952	Ultrafiltration	Renal Insufficiency Diabetes mellitus Intestinal obstruction Myocardial infarction	19	94.4 S.E. 1.3 94.2	93.9 S.E. 1.0 94.3
			Controls	16	S.E. 0.2	S.E. 0.2

* Percentages of diffusible serum sodium and potassium are calculated from the sodium and potassium concentrations of serum water and ultrafiltrate or dialysate water stated by the authors.

** Sodium and potassium concentrations were stated per liter of whole fluid, not per liter of water.

patients with renal insufficiency and diabetes mellitus. The mean percentages of ultrafiltrable sodium and potassium in the group of healthy subjects and the patient group did not show a statistically significant difference, but the standard error of the mean was greater in the patient group.

The effect of pathologic conditions on the composition of transsudation fluids formed by the body has been a subject of but little study. Loeb *et al.* (1922), Gollwitzer-Meier (1925), Hastings *et al.* (1926), Greene *et al.* (1931), Gilligan *et al.* (1934) and Folk *et al.* (1948) have, it is true, studied the distribution of sodium and potassium between plasma and fluids present in various diseases, i.e., edema fluid, ascites fluid and pleural effusion, but the object of these investigations was not to study the effect of the disease on the composition of the transsudate but to examine the distribution of these ions from a physiologic point of view, although the transsudates were drawn from patients, since these fluids are not readily obtainable from healthy persons. Tatum (1954), however, compared the sodium and potassium concentrations of edema fluid in toxemia of pregnancy and in nontoxemic pregnancy. The differences between the edema fluids in the two groups were small, but it nevertheless was seen that the sodium and potassium concentrations in the toxemia group were slightly higher than in the nontoxemia group, despite that the concentrations of these ions in the serum were lower in the toxemia group than in the control group.

Brink *et al.* (1959) studied the distribution of sodium and potassium into Dextran solution injected into the peritoneal cavity of normal and adrenalectomized rats. After equilibrium the sodium concentration of the Dextran solution was the same in adrenalectomized rats as in normal rats, with a few exceptions in which it was higher. The potassium concentration was higher in the peritoneal solution of adrenalectomized rats than in that of normal rats, but at the end of the experiment it was lower than the plasma potassium concentration, whereas in normal rats the potassium concentrations of the peritoneal fluid and plasma were equal.

It was found by van Oss *et al.* (1959) that addition of various synthetic detergents changed selectively the ultrafiltrabilities of sodium and potassium in an albumin solution. They stated that studies of the effect of substances of a more physiologic nature, such as steroids, cardiac glycosides, histamin, etc., are in progress.

OBJECT OF THE PRESENT INVESTIGATION

The study of factors influencing the diffusibility of sodium and potassium from the plasma is hampered by the difficulty of obtaining from the healthy body, for use as control material, a fluid that is ultrafiltrated or dialyzed from the plasma *in vivo*. A means to overcome this difficulty is to carry out the ultrafiltration across an artificial semipermeable membrane *in vitro*. As pointed out by Thorn (1960), because of the conflicting results of previous studies, further experiments of this kind are needed to clarify the state of alkali metal ions in serum.

It was the object to study at first, using certain "pure" protein solutions, the effect of the protein concentration, kind of protein, and pH of the solution on the ultrafiltrability of sodium and potassium. In the main part of this work, based on clinical cases, an attempt was made to answer the following guestions: Is there a correlation between changes in the serum proteins in various diseases and the ultrafiltrability of sodium and potassium, i.e., can changes in the Donnan factor in various diseases be observed experimentally? Do serum colloids adsorb sodium and potassium selectively? How is the potential difference between serum and its ultrafiltrate correlated to the ultrafiltrability of sodium and potassium? Does the pH of serum influence the ultrafiltrability of sodium and potassium? Doe the concentration of serum sodium and potassium correlate to the ultrafiltrability of these ions?

The plan of study for clarification of these questions was to carry out experiments on the ultrafiltrability of sodium and potassium from the serums of healthy persons and of patients with certain diseases in which marked disturbances occur in the sodium and potassium balance, the protein metabolism, and the regulation of the pH.

Study of the effect of aldosterone and heparin was also considered to be of interest, since the former is known to influence the distribution of sodium and potassium *in vivo* and the latter, in view of its physicochemical nature, might have an influence on the state of small ions.

MATERIAL AND METHODS

TEST SUBJECTS

Control Group.— The control group serums (table 7) were drawn from 27 apparently healthy members of the clinic staff. There were 14 males and 13 females aged 23 to 58 years, mean age 33.5 years.

Disease Groups.— The samples of serum and ascites fluid in the disease groups were obtained from patients in the following hospitals:

Kivelä Hospital (Helsinki municipal medical hospital): Patients No. 28 — 30, 32, 34 — 41, 49, 52 — 54, 57, 58, 64, 72, 74, 75, 77 — 90;

Laakso Hospital (Helsinki municipal tuberculosis hospital): Patients No. 59 — 63, 65 — 69, 73;

Maria Hospital (Helsinki municipal medical and surgical hospital): Patients No. 41, 55, 56, 91;

Second Medical Clinic, Central Hospital of the University of Helsinki: Patients No. 42, 43, 46, 47;

Hospital of the Wihuri Research Institute: Patients No. 45, 48;

Kiljava Tuberculosis Sanatorium: Patients No. 70, 71;

Second Surgical Clinic, Central Hospital of the University of Helsinki: Patients No. 31, 50;

Hesperia Hospital (Helsinki municipal hospital for mental diseases): Patient No. 44;

Hospital of Koskela Home for Aged: Patient No. 33.

The diagnoses of the patients in the following disease groups were made in the above mentioned hospitals. In selecting the patients for the investigation, the most representative cases of each disease were preferred.

Hepatic Cirrhosis Group. — The serum and ascites fluid samples in this group (table 8) were drawn from 24 patients with cirrhosis of the liver. The patients were 37 — 70 years of age, averaging

52.5 years. There were 17 males and 7 females. In the majority of cases, i.e., in 17 cases, the cirrhosis was of Laënnec type, while in 4 patients (No. 28, 36, 38 and 48) it was in part cardiac, in 2 patients (No. 47 and 50) postnecrotic, and in 1 patient (No. 29) obstructive biliary cirrhosis. The diagnosis has been confirmed by laparoscopy in 3 cases (No. 38, 41 and 50) and at autopsy in 4 cases (No. 29, 30, 35 and 51).

Nephrosis Group.— In the nephrosis group the serums (table 9) were taken from 6 patients with nephrosis, all of whom were edematous when the sample was drawn. The patients were aged 19—61 years, mean age 46.8 years. There were 2 males and 4 females. Patient No. 54 had proteinuria of 3 gm./24 hrs. and the others of 7—15 gm./24 hrs.

Pulmonary Tuberculosis Group.— The serums in this group (table 10) were obtained from 16 patients with advanced pulmonary tuberculosis and ranging in age from 34 to 62 years, mean age 52.5 years. There were 12 male and 4 female patients. All the patients in this group were in a poor physical condition with loss of weight, febrile temperature, and advanced tuberculous changes in the lungs.

Renal Insufficiency Group.— In the renal insufficiency group the serums (table 11) were taken from 18 patients with chronic renal insufficiency. There were 8 males and 10 females, 30—73 years of age, mean 53.5 years. In all cases the serum nonprotein nitrogen was over 80 mg. per 100 ml. or the creatinin was over 3 mg. per 100 ml. The highest level of nonprotein nitrogen was 161 mg. per 100 ml. in case 86. The most common cause of renal insufficiency was chronic glomerulonephritis or pyelonephritis. Four patients (No. 81, 84, 88 and 89) had for a long time used large amounts of analgesics containing phenacetin. Two patients (No. 74 and 86) had diabetic kidney disease.

PHYSICOCHEMICAL AND CHEMICAL METHODS

A blood sample of about 20 c.c. was drawn anaerobically under oil from the cubital vein with an injection syringe. Minimal stasis was used before inserting the needle into the vein. The samples were collected before the midday meal. The serum was separated by centrifugation at room temperature *c.* 1.5 hours after the sample was drawn.

Determination of the pH, ultrafiltration procedure, and measurement of the membrane potential were then carried out on the same day as the sample was drawn. Once or twice a week the serum water and the sodium, potassium and total protein concentrations were determined and electrophoretic protein fractionation was performed. In the meantime the samples were stored deep-frozen.

The *ultrafiltration* was carried out with the apparatus shown in fig. 1. It was constructed by using as the main body a pressure filtration apparatus, model MD 35—15, manufactured by the firm Membranfiltergesellschaft, Göttingen. The following changes were made in the original apparatus. Since the serum chamber and the supporting disc in the ultrafiltrate chamber were made of metals differing in their solubility in serum and ultrafiltrate, a potential difference of 200—250 mV was generated between the two metals. To prevent the resulting harmful effects of electrodialysis and corrosion, electric insulation between the serum and ultrafiltrate chambers was effected by substituting for the original ultrafiltrate chamber one

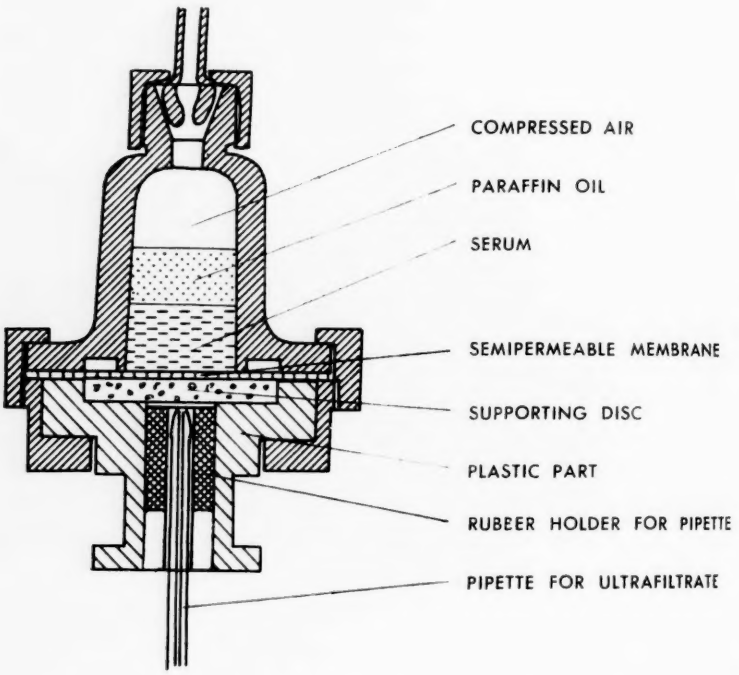

Fig. 1. — Apparatus for ultrafiltration.

made of nylon plastic. The structure of the chamber was modified in a manner to allow collection of the ultrafiltrate directly into a pipette whose point was close to the disc supporting the membrane. This arrangement was found to save time and material, since it allowed close recovery of the ultrafiltrate.

A cellulose dialyzing membrane (No. 4465—A2, Arthur H. Thomas Co., Philadelphia) was used. The membrane was 0.05 mm. in thickness and had an effective ultrafiltration area of 4 sq.cm.

When ultrafiltration was begun, 2.5 c.c. of serum was placed in the serum chamber and covered with a layer of paraffin oil to prevent exchange of gases between the serum and the compressed air. Three full pipettes of 0.2 ml. each of ultrafiltrate were usually taken for analysis, the contents of each pipette being analyzed separately. No clear differences were seen in the sodium and potassium concentrations of the successive samples, for in 20 ultrafiltrate experiments selected at random the mean sodium and potassium concentrations were 137.54 mEq./l. and 4.323 mEq./l. in the first pipette, 137.37 and 4.333 in the second pipette, and 137.38 and 4.310 in the third pipette. Augsberger (1928) and Laviates (1937) also stated that the composition of the ultrafiltrate was constant throughout the ultrafiltration process.

Under a pressure of 110 cm. Hg an aqueous physiologic solution of sodium chloride and potassium chloride passed across the dialyzing membrane at an even rate of 2.4 ml. per hour. On the other hand, the rate of ultrafiltration of serum was retarded with an increasing concentration of serum colloids onto the membrane. The average lenght of time required for formation of the first 0.2 ml. of ultrafiltrate form normal serum was 13 min., for the second 25 min., and for the third 30 min. The ultrafiltration rate of different serums varied greatly. The most rapid ultrafiltration occurred with certain serums with a low protein content in the pulmonary tuberculosis group. For example, the ultrafiltration rates of serums No. 58, 64 and 65 were 1.3—1.5 ml. during the first hour. The rates from normal serums were *c.* 0.5—0.9 ml during the first hour. Neither the total protein nor even the total dry substance concentration appeared to be the only factors with an effect on the ultrafiltration rate, for some of the serums in the nephrosis group (No. 53, 54 and 57) gave an ultrafiltrate yield of only 0.3—0.4 ml. during the first hour, although the total protein and the total dry substance concentrations in these

serums were below normal. They appeared to be highly lipemic, which may have contributed to the slow rate of ultrafiltration.

The *membrane potential* between serum and ultrafiltrate was measured after completed ultrafiltration by forcing ultrafiltrate from the serum across the dialyzing membrane and through a U-shaped capillary glass tube into a bulb at the end of one of the limbs, where the ultrafiltrate was brought into contact with a saturated KCl bridge leading to a calomel electrode (type K 400, Radiometer, Copenhagen). The serum was brought into contact with another saturated KCl bridge leading to an identical calomel electrode. The potential between the electrodes was measured with the potentiometer used for pH measurements.

The *sodium and potassium concentrations* were determined with a flame photometer (Leppänen *et al.* 1952) operating on the compensation principle. Lithium was used as the internal standard. The potassium was diluted 1:25 and the sodium 1:500. The sodium and potassium concentrations of each serum sample and of its ultrafiltrate were determined at the same time in duplicate. If the results of the double determinations differed more than 4 per cent, a third determination of sodium and potassium was made when the following set of serum and ultrafiltrate samples were tested, and the mean was calculated from the triplicate determinations.

It is known that the concentrations of serum alkali metals expressed per volume of serum may give a misleading picture of those concentrations per volume of serum water, since the volume of serum colloids, i.e., proteins and lipids, may vary markedly (Albrink *et al.* 1955). In order to calculate the sodium and potassium concentrations in *serum water* the latter was determined from the weight loss of a known volume of serum after it had been evaporated to dryness. The vessels used for evaporation were 5 ml. glass dishes made for flame photometer samples, the volume of serum for evaporation was 0.5 ml., and the time of evaporation 24 hours at 100° C.

It is open to question whether serum water determined by the gravimetric method represents the volume of water in which the ions are dissolved. Albrink *et al.* (1955) determined the serum water concentration also by determining the change in the freezing point of the serum after the addition of a given amount of sodium chloride

to a given volume of serum. This will enable calculation of the water volume in which the sodium chloride was dissolved. The result obtained conformed well with that obtained by the gravimetric method, which indicates that the serum water determined by the gravimetric method is "free" at least in the respect that its total amount serves as a diluent for added sodium chloride.

The water content of the ultrafiltrate was not measured but was assumed to be 99.0 gm. per 100 ml. (Tarail *et al.* 1952).

The *serum pH* measurement was made anaerobically at 38° C. using the apparatus described by Astrup and Schrøder (1956) and manufactured by Radiometer, Copenhagen. It was equipped with a combined glass and calomel electrode, model GK 264. The potential between the electrodes was measured with a precision potentiometer, model PHM 3, operating on the compensation principle. Standard buffer was Sørensen's phosphate buffer, containing 1.816 gm. of KH_2PO_4, 9.501 gm. of $Na_2HPO_4 \cdot 2H_2O$, and distilled water to make 1000 ml. Measured with a hydrogen electrode the pH of this buffer is 7.355 at 38° C (Radiometer, Copenhagen). After the pH measurement was completed the electrode chamber was rinsed with pepsin solution prepared with 0.1 N hydrochloric acid according to the instructions of Santz (1957) to clean the glass membrane and the contact point of the KCl bridge.

The *total protein* was determined by the biuret method, using the reagent of Gornall *et al.* (1949). The resulting color was measured with a Beckman B spectrophotometer using wavelength 540 mμ and the total protein was calculated from the readings by means of a standard curve.

Electrophoretic fractionations of the serum proteins were carried out by paper electrophoresis (Grassman and Hanning 1954), using veronal buffer pH 8.6 and ionic strength 0.06. After the run the fractions were dyed with amido black and the dried strips were rendered transparent with a mixture of paraffin oil and α-bromnaphthalin and were run in an extinction recorder (Carl Zeiss, Oberkochen). The relative percentages of the fractions were then determined planimetrically.

Ascites proteins were concentrated to be suitable for electrophoretic fractionation by forcing proteinfree ultrafiltrate from the ascites fluid.

CONTROL OF THE METHODS

The dialyzing *membrane* used for ultrafiltration was freely permeable to sodium and potassium ions. This was controlled by the filtration of an aqueous solution of sodium chloride and potassium chloride across the membrane. There was no difference in the sodium and potassium concentrations of the solution under filtration and those of the filtrate. No appreciable potential difference was seen between the two solutions. (Table 3.) The membrane used was therefore one of high porosity, in contrast to ion sieve membranes (Sollner 1954).

Table 3. — *Filtration of Sodium Chloride and Potassium Chloride Solutions across a Dialyzing Membrane*

Experiment No.	Solution before Filtration		Filtered Solution		Membrane Potential
	Na mEq./l	K mEq./l.	Na mEq./l.	K mEq./l.	mV
I	144.0	4.52	143.0	4.60	+2.0
II	142.0	4.00	143.5	4.00	0.0
III	132.0	3.75	132.5	3.75	−1.5
IV	139.0	4.05	138.0	4.00	+1.0

The membrane was not permeable to serum proteins, which was evident from the absence of turbidity in the ultrafiltrate upon addition of trichloroacetic acid or Spiegler's reagent.

The ultrafiltrate was always crystal clear in appearance, also in cases with icteric serum.

Concerning the effect of *heat*, Laviates (1937) stated that the amounts of ultrafiltrate formed by his method were as follows: 0.18 ml./h. at 7° C, 0.30 ml./h. at 27° C, and 0.37 ml./h. at 37° C.

In the present work the effect of heat on the rate of formation of ultrafiltrate and on the sodium and potassium contents of the ultrafiltrate was studied at room temperature (23°—25° C) and at 38° C (table 4). These experiments were performed with the serums of healthy subjects.

The sodium and potassium concentrations of ultrafiltrate formed at room temperature and at 38° C did not differ beyond the limits of methodical error. The rate of filtration at 38° C averaged 33 per cent more than at room temperature. Because of this higher rate of filtration, the actual ultrafiltration experiments were carried out in warm air at 38° C.

Table 4. — *Sodium and Potassium Concentrations and Rate of Formation of Serum Ultrafiltrate at Room Temperature (23°—25° C) and at 38° C*

Experiment No.	23°—25° C			38° C		
	$(Na)_f$ mEq./l. H_2O	$(K)_f$ mEq./l. H_2O	Rate of Ultrafiltration ml./hr.	$(Na)_f$ mEq./l. H_2O	$(K)_f$ mEq./l. H_2O	Rate of Ultrafiltration ml./hr.
1	140.0	4.10	0.48	138.5	4.08	0.66
2	145.0	4.30	0.49	145.0	4.31	0.61
3	133.0	4.20	0.68	135.0	4.22	0.91
4	134.0	4.25	0.47	140.0	4.30	0.65
Mean	138.6	4.17	0.54	138.8	4.17	0.72

Concerning the effect of *pressure* on the composition of serum ultrafiltrate. Burian (1909) observed that the freezing point of ultrafiltrate forced at very high pressures is higher than at low and moderate pressures. At pressures from 57 to 380 cm. Hg the freezing point of the ultrafiltrate was the same as that of the serum, but at 684 cm.Hg it was higher to the extent that its molality was 2.6 per cent lower than that of the serum. Grollman (1926) reported that the ultrafiltrability of calcium decreases with increasing filtration pressures. In the more recent literature on the ultrafiltrability of calcium, however, little attention has been paid to the possible effect of the ultrafiltration pressure.

Flexner (1937), on the other hand, stated that the ultrafiltrability of the chloride ion increases with increasing filtration pressures. Thus, for instance, the chloride concentration of ultrafiltrate made from a 7 per cent solution of casein was the same at that of the dialysis fluid when the filtration was carried out at a pressure of 40 cm.Hg, but 1.5 per cent higher at 140 cm.Hg and 2.2 per cent higher at 227 cm.Hg. According to Ambard and Trautmann (1960) the filtrability of sodium chloride decreases with increasing filtration pressures.

Table 5 gives a comparison of the sodium and potassium concentrations of serum ultrafiltrate formed at pressures of 18 cm.Hg and 110 cm.Hg. When the filtration was carried out at a pressure of 18 cm.Hg, 1—2 per cent more sodium and potassium passed across the membrane from nearly all the control and patient serums than at a pressure of 110 cm. Hg. The effect of pressure change on serums in the control group did not differ significantly from that in the hepatic cirrhosis group.

Table 5. — *A. Effect of Different Pressures on Sodium and Potassium Concentrations of Serum Ultrafiltrate*

Subjects	Serum		Ultrafiltrate at 18 cm Hg		Ultrafiltrate at 110 cm Hg	
	$(Na)_s$ mEq./l. H_2O	$(K)_s$ mEq./l. H_2O	$(Na)_f$ mEq./l. H_2O	$(K)_f$ mEq./l. H_2O	$(Na)_f$ mEq./l. H_2O	$(K)_f$ mEq./l. H_2O
Control	151.2	4.68	140.0	4.36	138.4	4.26
	156.5	4.94	150.0	4.22	147.0	4.20
	152.6	4.62	147.8	4.27	144.0	4.18
	159.0	4.97	146.0	4.28	143.0	4.23
Mean			146.0	4.29	143.1	4.22
Hepatic cirrhosis	139.5	3.20	135.1	3.12	132.3	3.09
	134.5	4.18	132.0	4.00	130.6	3.88
	145.5	3.11	137.0	2.99	135.6	2.94
	141.5	4.17	140.0	3.84	137.2	3.84
Mean			136.0	3.485	134.0	3.44
Pulmonary tuberculosis	150.8	4.88	144.2	4.43	141.6	4.30
	140.0	5.65	139.0	5.40	136.0	5.25
Nephrosis	150.0	5.08	144.0	4.91	142.5	4.89
	150.2	6.26	140.8	5.95	140.0	5.83
Renal insufficiency	155.5	4.90	150.0	4.36	147.0	4.28

B. *Statistical Comparison of Effect of Different Pressures on Sodium and Potassium Concentrations of Serum Ultrafiltrates in Control and Hepatic Cirrhosis Groups: t-Test*

	Na	K
Difference between means (per cent)	0.08	0.10
Combined mean error	0.373	0.782
t	0.214	0.128
P		

At 110 cm.Hg the rate of ultrafiltrate formation was about twice as rapid as at 18 cm.Hg. The pressure of 110 cm.Hg was therefore chosen for the ultrafiltration experiments.

The *methodical error* in the percentage of ultrafiltrable sodium and potassium was calculated by determining in duplicate the percentages of ultrafiltrable sodium and potassium in 10 serums and calculating the methodical error in the standard manner. The methodical errors were found to be 0.82 for the percentage of ultrafiltrable sodium and 0.71 for the percentage of ultrafiltrable potassium. The values of methodical error show that the error in

individual percentages of ultrafiltrability is in 95 per cent of cases smaller than plus or minus two times the methodical error.

ABBREVIATIONS AND STATISTICAL METHODS

In reporting the results the following expressions are used:

serum sodium concentration = Na_s mEq./l.;
serum sodium concentration in serum water = $(Na)_s$ mEq./l. H_2O:
ultrafiltrate sodium concentration in ultrafiltrate water = $(Na)_f$ mEq./l. H_2O:

percentage of ultrafiltrable serum sodium: $= \frac{100\,(Na)_f}{(Na)_s}$ %:

membrane potential, i.e., potential difference in millivolts between the ultrafiltrate and the serum = P_m. The sign − or + before a value indicates the kind of charge of the colloid fluid in relation to the ultrafiltrate.

The expressions for potassium (K) are analogous to those for sodium.

The results were tested by the usual statistical methods. Student's *t*-test was used for comparison of mean values. The method of least squares was applied to the regression analyses. The significance of the regression was calculated by the variance ratio (v^2) test. Statistical significance is designated by the symbol *P*, the value of which indicates the degree of probability by which the result may have been due to chance. If *P* was more than 0.5 this is indicated by two dots (..).

RESULTS

ULTRAFILTRABILITY OF SODIUM AND POTASSIUM AND MEMBRANE POTENTIAL OF CERTAIN PROTEIN SOLUTIONS

The ultrafiltrability of sodium and potassium and the membrane potential between the protein solution and its ultrafiltrate were studied using solutions of crystalized bovine serum albumin (Armour Laboratiories, Kankakee, Ill.) and of human albumin and

Table 6. — Ultrafiltrability of sodium and potassium and membrane potential in certain protein solutions

Experiment No.	Protein Solution	Protein Solution pH	Protein Solution (Na) mEq./l. H_2O	Protein Solution (K) mEq./l. H_2O	Ultrafiltrate (Na) mEq./l. H_2O	Ultrafiltrate (K) mEq./l. H_2O	100 · (Na) Ultrafiltrate % / (Na) Prot. Solution	100 · (K) Ultrafiltrate % / (K) Prot. Solution	P_m mV.
1	Bovine albumin 4 %	7.6	162.0	5.75	151.5	5.22	93.6	90.7	—4.5
2	..	3.0	151.0	6.30	162.0	6.49	107	103	+97.0
3	..	3.0	109.0	4.46	121.0	4.63	111	104	+30.0
4	..	3.0	79.0	2.24	94.0	2.38	119	106	+115.0
5	Human albumin 5 %	7.4	134.5	4.23	125.0	3.76	92.6	89.0	—9.0
6	.. 2.5 %	7.4	138.5	4.29	130.0	3.88	94.0	90.4	—2.0
7	.. 1.25 %	7.4	138.0	4.58	136.0	4.22	98.8	92.2	—1.0
8	Human γ-globulin 5 %	7.4	93.6	3.36	94.0	3.30	100	98.2	0.0
9	..	7.4	121.0	4.24	119.0	4.20	98.3	99.1	+1.0
10	Human albumin 5 % + human γ-globulin 2.5 %	7.4	142.0	3.58	130.0	3.10	91.6	86.6	—7.0

γ-globulin (Blood Service of the Finnish Red Cross, Helsinki). The results are shown in table 6.

In a 4 per cent bovine albumin solution and a 5 per cent human albumin solution at the physiologic pH the percentage of ultrafiltrable sodium was *c.* 93 per cent and that of potassium *c.* 90 per cent. The potential of the protein solution then was negative as compared with the ultrafiltrate (experiments 1 and 5). With decreasing albumin concentrations the percentage of filtrable cations increased and the membrane potential decreased (experiments 5, 6 and 7). Hydrochloric acid was added to the albumin solution in experiments 2, 3 and 4 to bring the pH to the acid side of the isoelectric point of albumin. The net charge of the albumin molecules then changes to positive, as a result of which more sodium and potassium was forced into the ultrafiltrate. Thus the concentration of sodium and potassium was higher on the ultrafiltrate side than on the albumin side, and the membrane potential became positive on the albumin side. These changes are in agreement with Donnan's theory.

In the experiments with γ-globulin (experiments 8 and 9) it was observed that at the physiologic pH the sodium and potassium are completely ultrafiltrable. Likewise, no noteworthy potential difference was measured between the γ-globulin solution and its ultrafiltrate.

EXPERIMENTS WITH SERUM

The results of experiments with serums from the control group and the various disease groups are presented in tables 7—11, which show the concentrations of sodium and potassium in serum, in serum water and in ultrafiltrate water, the percentages of ultrafiltrable serum sodium and potassium, the potential difference between serum and ultrafiltrate, the serum pH, and the serum total protein and its electrophoretic distribution. The group means, standard errors of the means, the differences between the means in the control group and each disease group, as well as the statistical significance of these differences (*P*) are also given.

Table 7. — *Control Group: Results of Analyses*

Subject No.	Sex	Age	Na_s mEq./l.	K_s mEq./l.	$(Na)_s$ mEq./l. H_2O	$(K)_s$ mEq./l. H_2O	$(Na)_f$ mEq./l. H_2O	$(K)_f$ mEq./l. H_2O	$\frac{100\ (Na)_f}{(Na)_s}$ %
1	M	24	147.0	4.65	157.0	4.97	146.6	4.32	93.2
2	M	34	147.0	3.89	157.0	4.15	143.0	3.64	91.5
3	F	23	143.0	4.59	152.0	4.87	137.0	4.26	90.3
4	F	40	138.0	4.40	147.0	4.69	137.0	4.15	93.0
5	F	33	143.5	4.46	152.0	4.73	140.0	4.12	92.1
6	M	28	138.0	4.24	148.0	4.54	139.5	3.97	94.0
7	M	26	145.0	4.42	156.0	4.74	144.5	4.16	92.8
8	M	28	143.0	3.69	153.0	3.96	141.0	3.40	92.0
9	M	29	148.0	4.62	159.0	4.97	143.0	4.23	89.9
10	F	39	143.5	4.50	153.5	4.81	140.0	4.26	91.3
11	M	25	145.5	4.79	155.0	5.11	147.0	4.68	94.5
12	M	28	145.5	4.42	156.0	4.75	148.0	4.36	94.6
13	M	28	138.0	3.76	148.0	4.05	138.0	3.55	93.7
14	F	43	142.0	4.52	152.0	4.84	142.0	4.30	93.5
15	F	42	138.5	4.87	147.5	5.19	135.5	4.72	91.8
16	M	58	145.0	4.92	155.0	5.25	144.0	4.80	93.1
17	F	35	143.0	4.45	153.5	4.78	142.0	4.25	92.4
18	F	30	141.0	4.42	151.0	4.74	138.5	4.27	91.5
19	F	53	138.0	4.35	147.5	4.65	139.5	4.14	94.5
20	F	23	137.5	4.10	147.0	4.39	138.0	3.90	93.6
21	F	53	143.0	4.33	152.5	4.62	144.0	4.18	94.5
22	M	27	146.0	4.61	156.5	4.94	147.0	4.20	94.0
23	F	32	146.0	4.81	156.0	5.14	147.0	4.45	93.4
24	F	27	141.0	4.49	150.5	4.79	140.5	4.30	93.4
25	M	29	144.5	4.65	155.0	4.98	143.0	4.45	92.3
26	M	40	136.0	4.64	145.5	4.95	139.0	4.48	95.5
27	M	32	142.0	4.84	152.0	5.17	138.5	4.50	91.3
Mean			142.6	4.46	152.4	4.77	141.5	4.22	92.9
Standard error of the mean			0.65	0.061	0.72	0.063	0.67	0.063	0.27

of Serums and Serum Ultrafiltrates

$\frac{100\,(K)_f}{(K)_s}$ %	Pm mV	Serum pH	Serum Total Protein gm. 100 ml.	Alb. %	Electrophoretic Distribution of Proteins α_1 %	α_2 %	β %	γ %
87.0	—8.0	7.37	7.1	62	2.4	7.6	12.0	16.0
87.7	—	7.41	6.8	61	2.2	9.2	11.5	16.0
87.5	—10.0	7.40	6.5	62	2.0	8.5	10.0	17.0
88.5	—	7.39	6.7	63	4.1	8.7	9.5	15.0
87.3	—11.0	7.38	6.3	62	3.0	8.5	11.5	15.0
87.5	—	7.38	7.15	60	5.2	14.0	9.5	12.0
87.8	—	7.41	7.3	63	2.6	6.7	11.0	16.5
85.9	—13.0	7.41	7.1	65	3.8	6.4	9.0	15.0
85.0	—	7.36	7.6	63	1.5	6.5	10.0	19.0
88.5	—14.0	7.36	7.0	63	1.4	4..	8.9	23.0
91.5	—9.5	7.42	6.5	52	3.8	13.0	13.7	17.5
91.9	—10.0	7.40	7.2	64	2.8	9.4	13.0	10.0
87.6	—7.5	7.38	7.1	68	2.8	5.6	9.9	14.0
88.9	—11.5	7.41	6.9	65	3.0	7.0	11.0	16.0
91.0	—10.0	7.34	6.7	52	4.8	8.3	11.5	24.0
91.5	—12.5	7.36	6.6	50	5.0	10.0	13.0	22.0
89.0	—10.0	7.41	7.5	50	3.6	9.5	12.4	25.0
90.2	—7.5	7.37	7.2	62	4.9	10.0	9.9	14.5
89.0	—7.0	7.37	6.8	57	4.5	11.5	11.4	16.0
89.0	—6.5	7.35	7.0	58	3.4	11.0	12.8	14.5
90.5	—9.0	7.42	6.6	60	3.1	10.5	11.4	14.5
85.0	—7.5	7.38	7.2	61	3.5	8.5	13.0	14.0
86.5	—9.0	7.38	6.8	51	3.7	8.1	12.0	25.0
89.8	—8.5	7.38	6.6	65	1.7	6.5	17.8	19.5
89.5	—7.0	7.38	7.0	56	4.5	9.5	10.7	19.0
90.5	—6.0	7.42	6.7	60	3.9	8.0	12.0	16.5
87.0	—9.0	7.36	6.9	61	4.2	7.8	12.6	14.5
88.6	—9.3	7.385	6.9	60	3.4	8.7	11.2	17.1
0.37	0.46	0.0044	0.059	0.96	0.21	0.42	0.29	0.74

Table 8. — *Hepatic Cirrhosis Group: Results of Analyses of Serums and Serum*

Patient No.	Sex	Age	Edema	Ascites	Na_s mEq. l.	K_s mEq. l.	$(Na)_s$ mEq. l. H_2O	$(K)_s$ mEq. l. H_2O	$(Na)_f$ mEq. l. H_2O	$(K)_f$ mEq./l. H_2O
28	M	56	+	+++	138.0	4.61	146.0	4.89	139.0	4.40
29	F	41	++	+	146.0	3.76	153.0	3.93	140.0	3.54
30a	M	60	+	+++	138.5	4.16	147.0	4.41	143.0	4.05
30b	M	60	+	+	138.5	3.86	146.0	4.08	141.0	3.74
31	M	50	+	++	144.5	3.25	153.0	3.45	143.0	3.12
32	M	70	++	++	134.5	3.80	142.0	4.02	138.5	3.80
33	M	55	—	—	132.5	4.21	141.0	4.50	134.0	4.27
34	F	59	+	—	130.5	2.96	139.5	3.16	132.5	3.10
35	F	61	+	+++	132.0	4.00	140.0	4.25	134.0	4.04
36	M	49	+++	+	136.0	3.83	142.0	4.00	136.5	3.85
37	M	66	—	—	136.0	4.40	144.0	4.67	141.0	4.35
38	M	39	—	—	134.0	3.92	141.5	4.13	131.0	3.88
39	M	55	—	—	133.5	5.05	143.0	5.42	136.5	4.85
40	M	54	—	+	136.0	2.91	145.0	3.11	136.0	2.94
41	M	47	—	—	133.0	4.34	142.5	4.65	135.0	4.35
42	M	72	—	+	138.0	3.95	147.0	4.20	135.5	3.86
43	F	60	—	—	138.0	4.42	148.0	4.73	142.0	4.37
44	M	67	+	+++	134.5	3.27	144.0	3.49	135.0	2.98
45	M	49	—	+	136.0	4.00	146.0	4.29	136.0	3.90
46	F	37	—	—	137.5	4.17	145.0	4.41	139.0	4.04
47	F	47	+	+	131.0	3.77	138.0	3.98	132.5	3.78
48	M	49	—	+	131.0	5.74	140.0	6.12	134.0	5.48
49	N	50	+	—	132.5	4.27	142.0	4.54	135.0	3.96
50	M	51	+	+	134.0	3.96	141.5	4.17	137.0	3.84
51	M	53	+	++	126.0	3.92	134.5	4.18	130.5	3.88
Mean					135.3	4.02	143.7	4.27	136.7	3.94
Standard error of the mean					0.85	0.12	0.84	0.13	0.72	0.11
Difference from control group					—7.25	—0.44	—8.77	—0.50	—4.87	—0.29
P					<0.001	<0.01	<0.001	<0.01	<0.001	<0.05

Table 9. — *Nephrosis Group: Results of Analyses of Serums and*

Patient No.	Sex	Age	Edema	Na_s mEq. l.	K_s mEq. l.	$(Na)_s$ mEq. l. H_2O	$(K)_s$ mEq. l. H_2O	$(Na)_f$ mEq. l. H_2O	$(K)_f$ mEq./l. H_2O
52a	M	52	+	140.0	4.09	147.5	4.31	140.0	3.94
52b	M	52	++	139.0	3.83	145.5	4.00	138.0	3.70
53a	F	61	++	140.5	4.79	150.0	5.10	145.0	4.73
53b	F	61	++	139.5	4.50	147.0	4.75	146.0	4.62
54	F	19	+++	132.5	4.04	139.0	4.24	137.5	4.03
55	F	46	++	137.0	4.11	145.0	4.36	140.5	4.14
56	M	44	+++	143.5	4.87	150.0	5.08	142.5	4.89
57	F	40	++	141.5	5.90	150.0	6.26	140.0	5.83
Mean				139.3	4.52	146.8	4.76	141.1	4.48
Standard error of the mean				1.2	0.24	1.2	0.26	1.1	0.24
Difference from control group				—3.36	0.06	—5.68	0.007	—0.35	0.26
P				<0.02	..	<0.001	..	..	..

Ultrafiltrates and Comparison with Results for Control Group

$\frac{100(Na)_f}{(Na)_s}$ %	$\frac{100(K)_f}{(K)_s}$ %	P_m mV.	Serum pH	Serum Total Protein gm./100 ml.	Alb. %	Electrophoretic Distribution of Proteins α_1 %	α_2 %	β %	γ %
95.0	90.0	—3.9	7.36	5.7	43	6.1	9.5	15	27
91.6	90.1	—3.5	7.35	4.2	37	1.6	7.2	14	40
97.3	91.8	—2.5	7.43	5.8	34	4.0	4.3	8.6	49
96.6	91.6	—3.5	7.48	5.1	35	5.4	4.0	9.0	47
93.4	90.5	—3.0	7.43	5.8	36	7.3	11.3	7.3	38
97.5	94.5	—0.5	7.49	5.7	39	5.3	5.3	8.5	42
94.9	95.0	—5.5	7.33	7.0	28	3.6	8.9	13	46
94.9	98.0	—9.0	7.48	7.1	31	5.4	9.0	14	41
95.6	95.1	—5.5	7.53	6.3	29	4.9	9.0	13	44
96.0	96.2	—10.0	7.40	4.0	27	4.5	8.4	12	48
97.5	93.1	—6.5	7.40	5.6	53	2.3	8.1	13	24
93.0	93.8	—14.0	7.45	5.1	51	3.9	7.3	12	25
95.5	89.4	—3.0	7.34	7.2	38	4.4	10.5	18	29
93.5	94.5	—6.0	7.37	6.6	35	5.3	6.9	11	41
94.9	93.6	—6.5	7.47	6.9	48	3.6	10.0	14	25
92.5	92.0	—10.0	7.40	6.1	42	4.6	9.2	12	31
95.8	92.4	—4.0	7.45	7.0	41	4.5	9.1	14	31
94.0	85.5	—5.0	7.48	6.8	39	7.2	10.0	17	26
93.0	92.4	—7.0	7.43	7.4	47	5.4	9.2	12	26
95.8	91.5	—5.0	7.38	5.3	49	4.2	8.3	10	28
95.9	95.1	—5.5	7.44	5.5	38	8.0	10.0	11	33
95.7	89.5	—15.0	7.36	6.6	32	6.4	12.0	16	34
95.0	87.2	—8.5	7.44	7.3	43	7.5	13.0	13	23
97.2	92.1	—2.0	7.45	5.3	41	5.8	8.4	12	33
97.1	92.9	—4.0	7.46	6.3	38	5.3	7.1	7.2	42
95.2	92.3	—6.0	7.42	6.2	39	5.1	8.6	12	35
0.33	0.56	0.72	0.011	0.19	1.4	0.31	0.45	0.56	1.4
2.29	3.75	—(—3.31)	0.03						
<0.001	<0.001	<0.001	<0.02						

Serum Ultrafiltrates and Comparison with Results for Control Group

$\frac{100(Na)_f}{(Na)_s}$ %	$\frac{100(K)_f}{(K)_s}$ %	P_m mV.	Serum pH	Serum Total Protein gm./100 ml.	Alb. %	Electrophoretic Distribution of Proteins α_1 %	α_2 %	β %	γ %
95.0	91.4	—7.0	7.38	4.8	23.0	6.1	22.0	18.0	31.0
94.6	92.6	—3.5	7.45	3.7	18.0	9.7	26.0	19.0	27.0
96.8	92.6	—4.0	7.36	6.5	39.5	5.3	31.5	10.0	14.0
99.1	97.3	—	7.40	5.2	28.0	7.6	20.0	25.0	19.0
98.8	95.0	—	7.30	4.3	15.0	9.0	41.0	19.0	16.0
96.6	95.0	—7.5	7.38	4.8	11.0	8.6	33.0	14.5	33.0
95.3	95.6	—7.0	7.34	3.5	42.5	4.4	21.0	18.0	14.0
93.1	93.1	—10.0	7.16	5.9	20.5	11.0	40.0	14.0	15.0
96.2	94.1	—6.5	7.35	4.8	24.7	7.7	29.3	19.6	21.1
0.73	0.69	0.98	0.031	0.36	4.0	0.81	3.4	1.5	2.8
3.28	5.52	—(—2.77)	—0.04						
<0.001	<0.001	<0.02	..						

Table 10. — *Pulmonary Tuberculosis Group: Results of Analyses of Serums*

Patient No.	Sex	Age	Na_s mEq./l.	K_s mEq./l.	$(Na)_s$ mEq./l. H_2O	$(K)_s$ mEq./l. H_2O	$(Na)_f$ mEq./l. H_2O	$(K)_f$ mEq./l. H_2O	$\frac{100(Na)_f}{(Na)_s}$ %
58	M	57	126.0	4.26	134.0	4.54	129.5	3.93	96.8
59	M	34	132.0	4.60	138.5	4.84	138.0	4.62	99.4
60	M	54	141.5	5.70	150.0	6.04	142.0	5.21	94.5
61	F	51	132.0	4.57	141.0	4.88	138.0	4.50	98.0
62	M	53	135.0	4.62	144.0	4.95	140.5	4.49	97.4
63	F	53	111.5	5.36	118.5	5.68	111.5	4.73	94.2
64	F	54	133.0	4.85	140.0	5.12	135.0	4.50	96.2
65	M	60	135.0	4.35	144.0	4.64	141.0	3.91	98.0
66	M	43	141.5	4.54	149.0	4.79	141.0	4.30	94.5
67	M	36	138.0	5.58	145.0	5.86	137.5	5.30	94.6
68	M	61	138.0	4.50	147.0	4.79	140.0	4.26	95.2
69	M	62	135.5	5.09	145.0	5.43	138.5	4.75	95.5
70	M	50	131.0	5.31	144.5	5.65	136.0	5.25	94.2
71	M	49	138.0	4.64	149.0	5.01	142.0	4.53	95.5
72	F	59	138.0	4.59	146.0	4.87	139.5	4.32	95.4
73	M	61	133.0	4.86	141.5	5.16	139.5	4.63	98.6
Mean			133.7	4.84	142.3	5.14	136.8	4.58	96.1
Standard error of the mean			1.8	0.11	1.9	0.11	1.8	0.10	0.42
Difference from control group			—8.87	0.378	—10.10	0.371	—4.75	0.353	3.25
P			<0.001	<0.01	<0.001	<0.01	<0.02	<0.01	<0.001

Table 11. — *Renal Insufficiency Group: Results of Analyses of Serums and*

Patient No.	Sex	Age	Na_s mEq./l.	K_s mEq./l.	$(Na)_s$ mEq./l. H_2O	$(K)_s$ mEq./l. H_2O	$(Na)_f$ mEq./l. H_2O	$(K)_f$ mEq./l. H_2O	$\frac{100(Na)_f}{(Na)_s}$ %
74	F	73	144.0	5.19	151.5	5.45	145.0	5.20	95.6
75	M	57	137.0	5.38	144.0	5.66	136.5	5.23	94.5
76	M	53	139.5	4.97	148.5	5.29	145.5	4.74	98.0
77	F	58	134.5	3.74	142.5	3.97	138.5	3.62	97.1
78	M	57	130.0	8.80	138.0	9.34	128.5	8.48	93.2
79	M	62	146.5	4.64	157.5	4.99	148.0	4.38	94.1
80	F	59	138.0	4.75	147.5	5.11	142.0	4.37	96.3
81	M	64	145.0	5.02	154.0	5.34	147.5	4.75	96.0
82	F	60	132.0	4.52	140.5	4.80	130.5	4.59	93.0
83	F	58	140.5	5.79	149.0	6.14	140.5	5.25	94.1
84	F	39	136.0	2.59	143.5	2.73	135.0	2.55	94.1
85	M	52	136.0	6.04	144.0	6.40	138.5	5.73	96.0
86a	M	30	136.5	6.72	145.0	7.13	131.0	6.28	90.5
86b	M	30	135.5	5.98	143.0	6.32	134.0	5.71	94.0
87	F	56	128.0	6.11	136.0	6.52	129.5	5.89	95.1
88	F	68	145.0	4.46	155.5	4.90	147.0	4.28	94.5
89	F	56	137.0	3.80	145.5	3.80	130.5	3.55	90.4
90	F	41	131.5	7.10	142.0	7.65	132.5	6.68	93.5
91	M	54	127.0	5.27	136.0	5.63	129.5	5.30	95.2
Mean			136.9	5.31	145.4	5.64	137.4	5.08	94.5
Standard error of the mean			1.3	0.31	1.4	0.16	1.6	0.30	0.44
Difference from control group			—5.73	0.85	—7.04	0.871	—4.16	0.859	1.61
P			<0.001	<0.02	<0.001	<0.001	<0.02	<0.01	<0.01

and Serum Ultrafiltrates and Comparison with Results for Control Group

$100\frac{(K)_f}{(K)_s}$ %	P_m mV.	Serum pH	Serum Total Protein gm./100 ml.	Alb. %	Electrophoretic Distribution of Proteins α_1 %	α_2 %	β %	γ %
86.5	—5.0	7.41	6.3	39.5	7.2	12.0	13.5	28.0
95.5	—5.0	7.36	4.8	31.0	7.4	36.0	11.0	14.0
86.4	—	7.32	5.7	16.5	6.6	34.0	17.0	26.0
92.3	—3.9	7.38	6.6	30.0	6.0	24.0	17.0	23.0
90.7	—7.7	7.34	7.0	42.0	5.2	9.5	8.4	35.0
83.3	—5.0	—	5.6	23.0	9.2	16.5	18.5	32.5
87.9	—9.0	7.40	5.5	—	—	—	—	—
84.3	—6.5	7.36	6.5	46.0	3.4	10.0	15.0	26.0
89.8	—5.5	7.38	4.9	16.5	2.2	35.5	16.5	29.0
90.4	—8.0	7.26	4.7	42.0	5.0	15.0	12.0	26.0
89.0	—3.0	7.45	6.3	35.0	7.8	21.0	16.0	21.0
87.5	—6.0	7.34	6.5	43.0	4.6	12.0	14.0	27.0
92.9	—5.0	7.22	6.2	40.5	11.0	22.0	11.0	16.0
90.5	—2.5	7.23	7.5	42.0	4.8	12.5	15.5	28.5
88.7	—3.0	7.40	5.9	61.0	3.7	8.5	12.0	15.0
89.6	—7.5	7.41	6.0	36.0	8.7	16.0	9.5	30.0
89.1	—5.5	7.35	6.0	36.3	6.2	18.6	13.4	24.9
0.78	0.51	0.18	0.20	3.0	0.62	2.6	0.85	1.7
0.52	—(—3.76)	—0.04						
"	<0.001	<0.05						

Serum Ultrafiltrates and Comparison with Results for Control Group

$100\frac{(K)_f}{(K)_s}$ %	P_m mV.	Serum pH	Serum Total Protein gm./100 ml.	Alb. %	Electrophoretic Distribution of Proteins α_1 %	α_2 %	β %	γ %
95.5	—2.8	7.28	4.8	57	9.2	5.5	12.0	16.5
82.5	—6.5	7.22	4.9	—	—	—	—	—
89.6	—11.0	7.29	6.4	53	4.0	13.5	8.0	21.0
91.2	—	7.41	6.0	54	4.7	12.5	9.5	19.0
90.8	—	7.08	6.1	43	9.5	13.5	12.0	22.0
87.9	—7.0	7.40	7.0	55	5.6	15.5	14.5	9.5
85.5	—12.0	7.35	6.9	52	4.5	9.0	10.0	25.0
89.0	—8.0	7.27	6.1	69	2.0	10.5	11.5	7.0
95.5	—	7.23	6.1	51	3.4	13.5	10.5	21.0
85.6	—6.5	7.31	6.2	49	5.1	14.5	18.0	14.5
93.5	—7.0	7.43	4.8	59	8.1	8.1	12.0	12.6
89.5	—7.5	7.19	5.6	39	9.7	16.0	17.0	17.0
88.0	—6.5	7.26	5.7	36	9.1	28.0	10.0	16.0
90.4	—6.8	7.32	5.2	36	7.5	30.0	11.0	15.6
90.3	—8.0	7.18	6.4	51	8.8	12.0	11.0	17.5
87.4	—7.5	7.13	7.0	—	—	—	—	—
93.5	—1.5	7.23	5.1	41	9.2	14.0	9.2	26.0
87.3	—15.0	7.28	7.3	59	9.4	8.8	7.5	15.0
94.0	—2.0	7.33	6.7	25	9.6	12.0	13.0	40.5
90.4	—7.23	7.27	6.0	49	7.0	13.9	11.6	18.6
0.71	0.87	0.021	0.18	2.6	0.66	1.5	0.69	1.8
1.81	—(—2.04)	—0.12						
<3.01	0.05	0.001						

Ultrafiltrability of Sodium and Potassium and Membrane Potential in the Various Groups

From the results shown in tables 7—11 it is seen that the mean *percentage of ultrafiltrable serum sodium* was 92.9 per cent in the control group, 95.2 per cent in the hepatic cirrhosis group, 96.2 per cent in the nephrosis group, 96.1 per cent in the pulmonary tuberculosis group and 94.5 per cent in the renal insufficiency group. The percentages of ultrafiltrable sodium were significantly higher in all the disease groups than in the control group. The mean *percentage of ultrafiltrable serum potassium* was 88.6 per cent in the control group, 92.2 per cent the hepatic cirrhosis group, 94.1 per cent in the nephrosis group, and 90.4 per cent in the renal insufficiency group. The percentages of ultrafiltrable potassium were significantly higher in the disease groups than in the control group with the exception of pulmonary tuberculosis.

Since the percentage of ultrafiltrable sodium was slightly higher than that of ultrafiltrable potassium in most of the serums, the mean ratio of the percentages of ultrafiltrable sodium and ultrafiltrable potassium was greater than one in all the groups. However, significant differences were seen in these rations in the various groups. The ratio

Table 12. — *A. Mean Ratios: Percentage of Ultrafiltrable Sodium/Percentage of Ultrafiltrable Potassium in Different Groups*

	Control	Hepatic Cirrhosis	Nephrosis	Pulmonary Tuberculosis	Renal Insufficiency
Mean ratio	1.0491	1.0317	1.0223	1.0801	1.0468
Standard error of the mean	0.00418	0.00665	0.00640	0.00932	0.00992

B. Statistical Comparison of Ratios in A.: t-Test

Compared Groups	Difference between Means	Combined Mean Error	*t*	*P*
Control — Hepatic cirrhosis	0.0174	0.007856	2.21	<0.05
Control — Pulmonary tuberkulosis	—0.0310	0.01021	3.04	<0.01
Control — Nephrosis	0.0268	0.007642	3.51	<0.01
Control — Renal insufficiency	0.0023	0.01077	0.21	..
Hepatic cirrhosis — Pulmonary tuberculosis	—0.0484	0.01145	4.23	<0.001

in the pulmonary tuberculosis group, on the one hand, and the hepatic cirrhosis group and the nephrosis group, on the other hand, changed in diverging directions, so that, in relation to the ultrafiltrability of sodium, little potassium was ultrafiltrable in the pulmonary tuberculosis group, while in the hepatic cirrhosis and nephrosis groups a high percentage of potassium was ultrafiltrable. In this respect the control and renal insufficiency groups were intermediate between the above groups (table 12).

The *membrane potential* between the ultrafiltrate and the serum was —9.3 mV in the control group. The potential of the serum in relation to the ultrafiltrate was clearly negative in all the serums in the control group, the smallest potential difference being —6.0 mV (serum No. 26). The potential differences between the serum and the ultrafiltrate in the disease groups were significantly smaller than in the control group (tables 7—11). In certain patient serums, chiefly

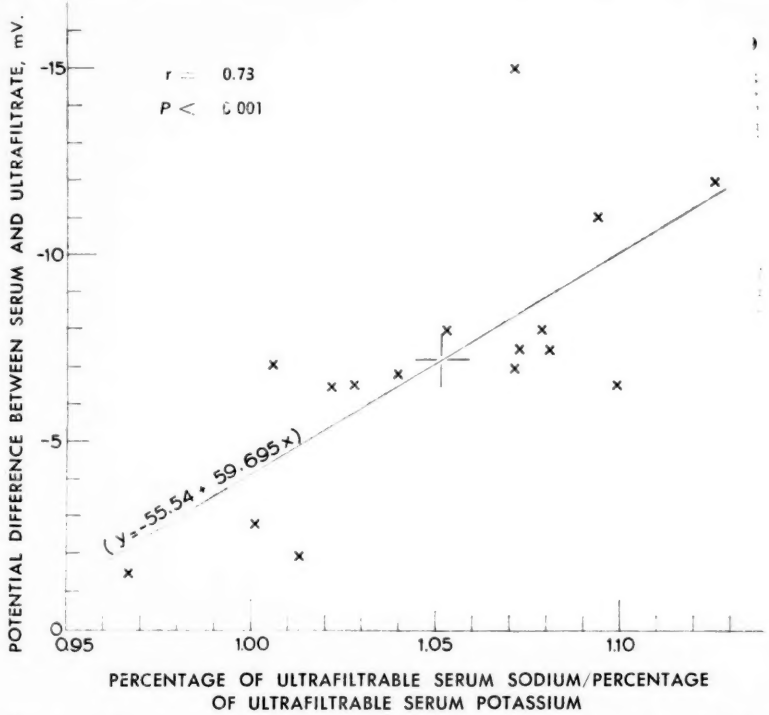

Fig. 2. — Correlation of membrane potential between serum and ultrafiltrate to selective serum adsorption of potassium in preference to sodium (ratio: percentage of ultrafiltrable serum sodium/percentage of ultrafiltrable serum potassium) in renal insufficiency group.

in the hepatic cirrhosis group, the membrane potential was only —0.5 to —3.0 mV (No. 30, 31, 32, 39 and 50). However, no serum was positively charged when measured against the ultrafiltrate.

Ling (1952) has advanced the hypothesis that it is the membrane potential that influences the selective distribution of sodium and potassium between body fluids. According to his hypothesis, potassium is accumulated in preference to sodium in the negatively charged side of the membrane due to the physical differences between these ions. To study in the present work the possible relationship between the quantities of membrane potential and selective adsorption of potassium, these variables were plotted against each other. It was observed, however, that a correlation exists only in the renal insufficiency group, in the respect that with increasing membrane potentials the selective adsorption of potassium (ratio: percentage of ultrafiltrable sodium/percentage of ultrafiltrable potassium) also increases (fig. 2).

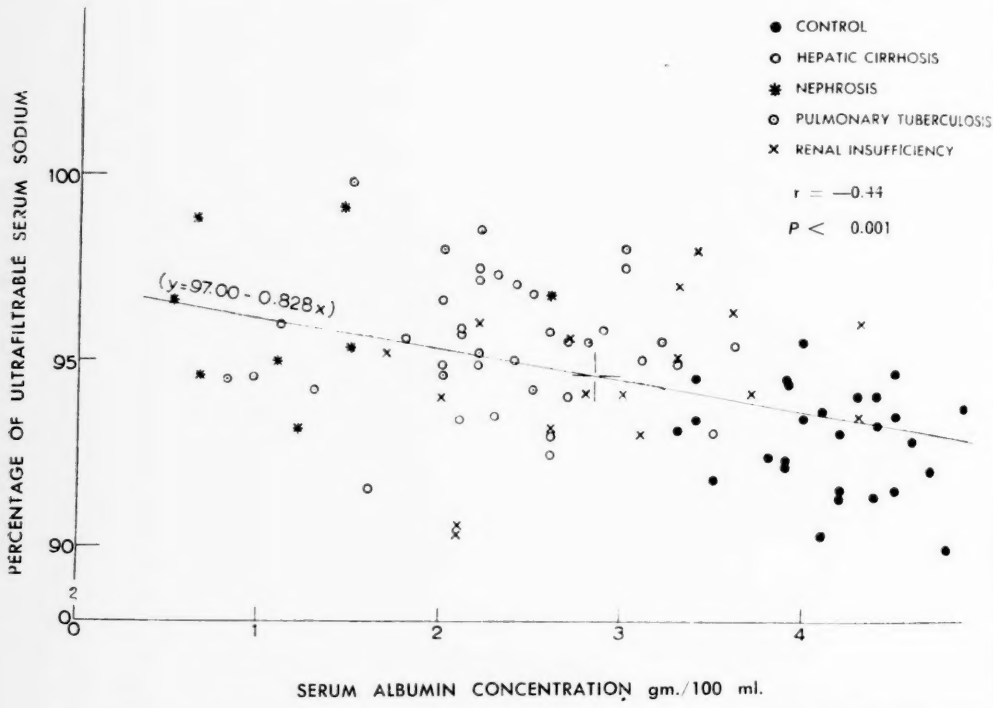

Fig. 3. — Correlation of serum albumin concentrations to percentages of ultrafiltrable serum sodium in all cases.

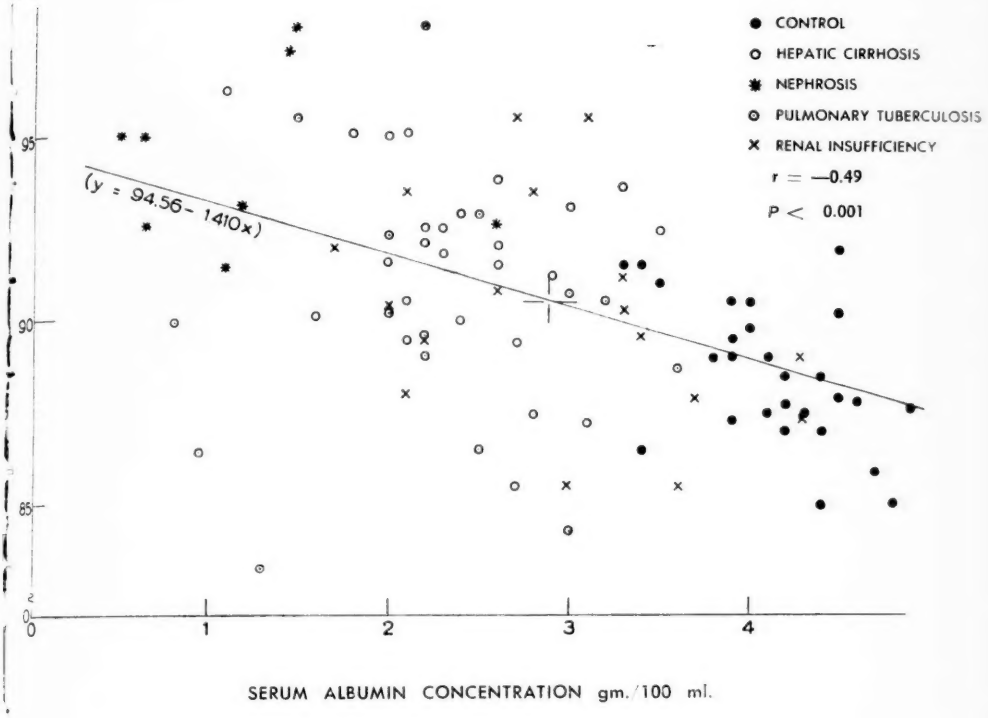

Fig. 4. — Correlation of serum albumin concentrations to percentages of ultrafiltrable serum potassium in all cases.

Correlation of Ultrafiltrability of Serum Sodium and Potassium to Serum Albumin Concentration

The serum albumin concentration was plotted against the percentage of ultrafiltrable serum sodium in all cases (fig. 3). Similarly, the serum albumin concentration was plotted against the percentage of ultrafiltrable serum potassium (fig. 4).

It can be seen from figs. 3 and 4 that the ultrafiltrabilities of serum sodium and serum potassium increase with decreasing serum albumin concentrations.

Cation Equivalency of Serum Proteins and Colloids and Correlation of Latter to Serum Sodium Concentration

The cation equivalency of serum proteins can be calculated from the albumin and globulin concentrations and the pH according to the formula of Van Slyke (p. 11, equation 7). The cation equivalency of the all serum colloids can be calculated by the Donnan equation (p. 9, equation 2) on the basis of the ultrafiltrability of sodium. The cation equivalencies of serum proteins and serum colloids in each group calculated in these two manners are given in table 13. The group means were used in these calculations. It is seen from table 13 that the cation equivalency both of serum proteins and of serum colloids in the disease groups is smaller than in the control group. Peters *et al.* (1929) and Broch (1945) have also reported that the "base binding power of serum proteins" is lowered in liver, kidney and lung diseases.

Table 13. — *Mean Cation Equivalency of Serum Proteins Calculated by the Formula of Van Slyke and Mean Cation Equivalency of Serum Colloids Calculated according to Donnan's Theory in Different Groups.*

Group	Serum Proteins mEq./l. H_2O Van Slyke	Serum Colloids mEq./l. H_2O Donnan
Control	18.0	21.1
Hepatic cirrhosis	14.9	13.7
Nephrosis	10.7	10.5
Pulmonary tuberculosis	14.0	11.1
Renal insufficiency	14.2	15.4

A correlation can be seen, on the whole, between the cation equivalency of serum colloids calculated from the ultrafiltrability of sodium according to Donnan's equation and the serum sodium concentration in the respect that the serum sodium concentration decreased with decreasing cation equivalency of serum colloids (fig. 5).

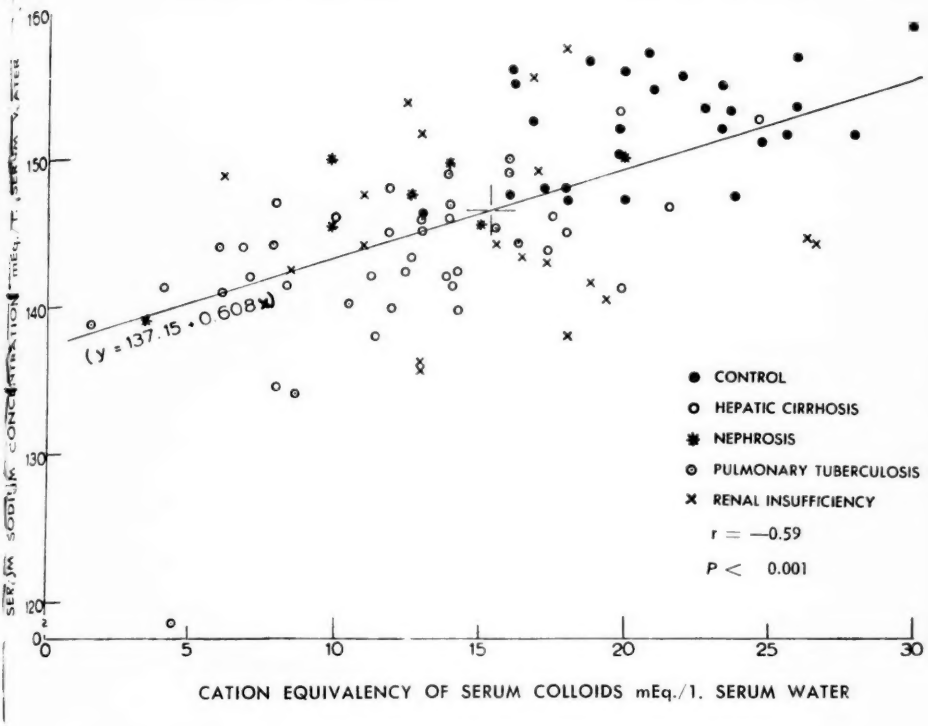

Fig. 5. — Correlation of serum sodium concentrations to cation equivalencies of serum colloids in all cases.

Correlation of Ultrafiltrability of Serum Sodium and Potassium to Concentration of These Ions

To study how the percentage of ultrafiltrable serum sodium is related to the sodium concentration in serum water, these variables were plotted against each other. It is seen that the percentage of ultrafiltrable serum sodium increased with a decreasing sodium concentration (fig. 6).

A tendency to a similar correlation was noted between the potassium concentration of serum water and the percentage of ultrafiltrable serum potassium, but this correlation (fig. 7) was not as significant as in the case of sodium.

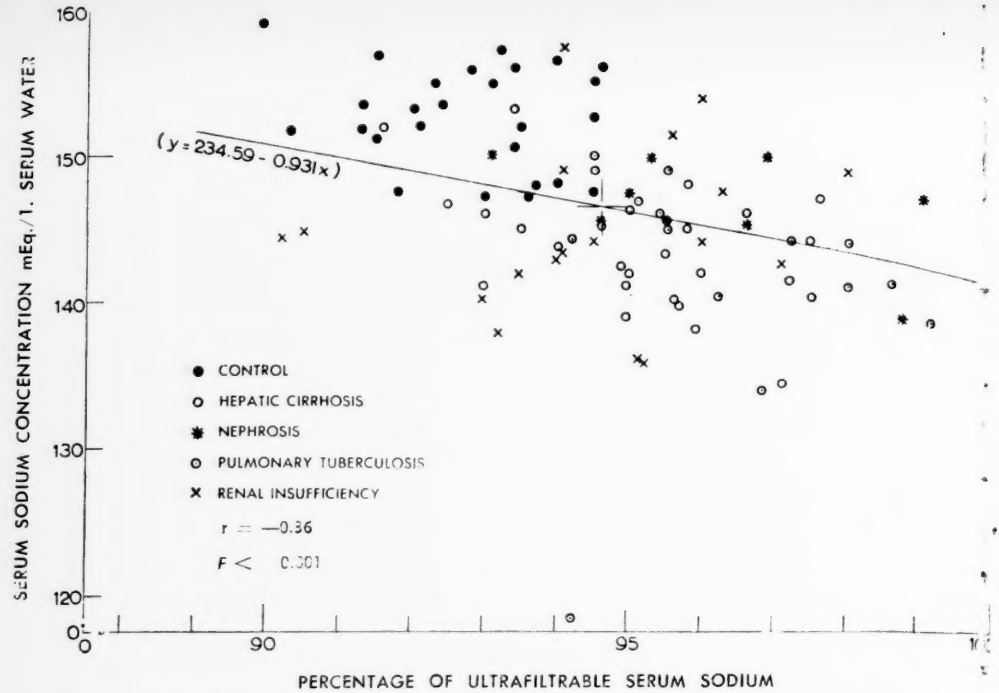

Fig. 6. — Correlation of serum sodium concentrations to percentages of ultrafiltrable serum sodium in all cases.

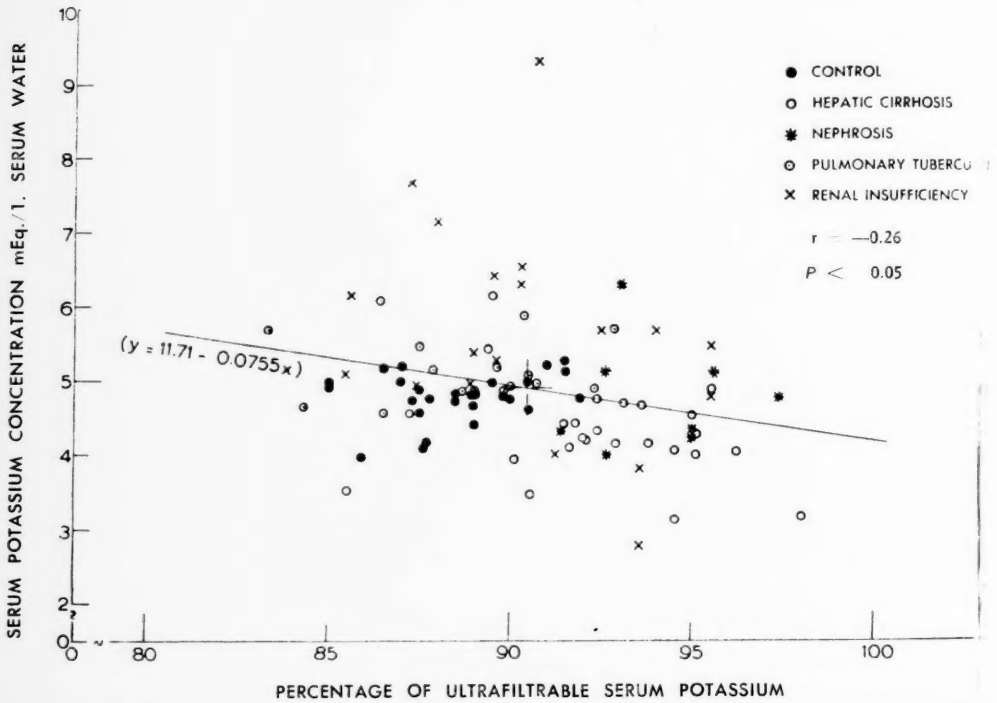

Fig. 7. — Correlation of serum potassium concentrations to percentages of ultrafiltrable serum potassium in all cases.

Serum pH and Its Correlation to Serum Potassium Concentration

The mean pH of serum was 7.385 in the control group. In the nephrosis group it was 7.346. which does not significantly differ from the control group. In the pulmonary tuberculosis group the mean pH was 7.350. i.e.. slightly lower than in the control group. The low pH value seen in the renal insufficiency group. pH 7.273. is in agreement with the values in the survey published by Møller (1959) and obtained in his own investigations. The hepatic cirrhosis group had the highest pH value in the present series. being 7.420. An increasing number of studies have been published recently giving evidence that patients with cirrhosis of the liver frequently have respiratory alkalosis (e.g.. Vanamee *et al.* 1956. Møller 1959. Heineman *et al.* 1960).

The total CO_2 content of plasma was determined by the clinical laboratory for 5 patients with cirrhosis of the liver in the present series. and in these cases the pCO_2 could therefore be calculated. This was done with the aid of a table (Møller 1959) based on the equation of Henderson-Hasselbalch.

Table 14. — *Venous pH. Total CO_2 and pCO_2 of Five Patients with Hepatic Cirrhosis*

Patient No.	pH	Total CO_2 mEq./l.	pCO_2 mm. Hg
31	7.43	23	34
32	7.49	22	29
34	7.48	26	35
35	7.53	25	30
40	7.37	13	22

The normal pCO_2 is slightly over 40 mm.Hg (e.g.. Møller 1959) and therefore the pCO_2 of the 5 patients with hepatic cirrhosis displayed a tendency to be low (table 14). This agrees well with earlier reports that the alkalosis present in cirrhosis of the liver is respiratory in origin.

Leibman and Edelman (1959) have shown that a correlation can be seen between the serum pH and the serum potassium concentration in a heterogeneous series of patients in the respect that the potassium concentration decreases with increasing pH values. A similar correlation can be seen in the present work (fig. 8).

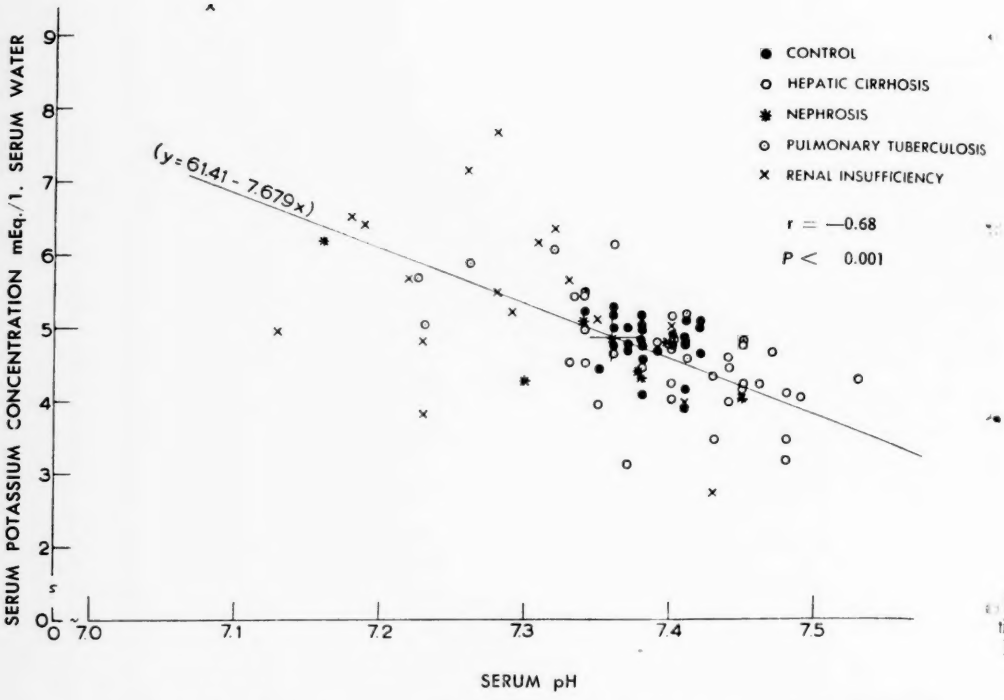

Fig. 8. — Correlation of serum pH levels to serum potassium concentrations in all cases.

Comparison of Results of Serum, Ascites Fluid and Serum Ultrafiltrate Analyses

After taking of a blood sample for ultrafiltration, ascites fluid was obtained by paracentesis from 5 patients with cirrhosis of the liver (No. 28, 30, 31, 32 and 35). Table 15 shows the results of the analyses made of the serum, ascites fluid and serum ultrafiltrate of these patients. It is seen that the *sodium concentrations* of the ascites fluid and the serum ultrafiltrate were of the same order of magnitude. On the other hand, some differences were seen between the *potassium concentration* of the ascites fluid and that of the serum ultrafiltrate. An explanation may be provided by the fact that transfer of potassium occurs between the serum and the blood cells after the blood sample is drawn. Consequently the serum potassium concentration does not give an accurate picture of the potassium concentration of the plasma with which the ascites fluid was in

equilibrium. Therefore, this is a disturbing factor in the determination of the diffusibility of potassium *in vivo* (Folk *et al.* 1948, Gerbrandy *et al.* 1960).

The *pH* of the ascites fluid was measured by the same method as that of serum, i.e., anaerobically at 38° C. In the examined cases the ascites fluid was slightly more alkaline than the serum, the difference in pH averaging 0.07 pH unit (table 15). The membrane potential between serum and ascites fluid was calculated on the basis of their hydrogen ion activities (p. 10, equation 5). The calculated mean membrane potential between serums and ascites fluids was —4.6 mV. The mean measured membrane potential between the same serums and their ultrafiltrates was —3.1 mV (table 15).

The total *protein concentration* of ascites fluid was 0.4 — 3.0 gm. per 100 ml. On comparison of the electrophoretic fractionation of pro-

Table 15. — *Results of Analyses of Serum, Ascites Fluid and Serum Ultrafiltrate of Five Patients with Hepatic Cirrhosis*

Patient No.	Fluid	(Na) mEq./l. H_2O	(K) mEq./l. H_2O	pH	Measured P_m mV. Ultrafiltrate—Serum	Calculated P_m mV. Ascites Fluid—Serum	Total Protein gm./100 ml.	Alb. %	Electrophoretic Distribution of Proteins α_1 %	α_2 %	β %	γ %
									Per Cent of Total			
cases. 28	Serum	146	4.89	7.36	—3.9	—8.0	5.7	43	6.1	9.5	15.	27
	Ascites fluid	143	3.52	7.49			3.0	37	8.5	11.5	16.5	28
	Ultrafiltrate	139	4.40									
30	Serum	147	4.41	7.43	—2.5	—0.6	5.8	34	4.0	4.3	8.6	49
	Ascites fluid	143	4.22	7.44			0.7	39	3.5	2.8	11.2	43
	Ultrafiltrate	143	4.05									
31	Serum	153	3.45	7.43	—3.0	—3.1	5.8	36	7.3	11.4	7.3	38
	Ascites fluid	144	3.32	7.48			1.8	32	7.2	7.9	7.2	45
	Ultrafiltrate	143	3.10									
32	Serum	142	4.02	7.49	—0.5	—2.5	5.7	39	5.3	5.3	8.5	42
	Ascites fluid	137	3.78	7.53			1.5	42	5.8	4.3	8.7	39
	Ultrafiltrate	138	3.80									
35	Serum	140	4.25	7.53	—5.5	—8.7	6.4	29	4.9	9.0	12.8	44
	Ascites fluid	133	3.80	7.67			0.4	37	6.3	7.1	12.0	38
	Ultrafiltrate	134	4.04									

teins in serum and in ascites fluid (table 15) a difference was found in patients No. 30, 32 and 35 in the respect that the ascites fluid protein was composed relatively more of albumin and less of α_2-globulin and γ-globulin than the serum protein. Wiederman and Šmarda (1958) have demonstrated that such a distribution of proteins occurs also across an artificial membrane into the serum filtrate when the pores of the membrane are large enough to permit some of the serum proteins to pass through. These differences were not seen in cases 28 and 31, in which the total protein concentrations of the ascites fluid and the serum did not differ as greatly as in the cases mentioned above.

EFFECT OF ALDOSTERONE AND HEPARIN ON ULTRAFILTRABILITY OF SODIUM AND POTASSIUM *IN VITRO*

Effect of Aldosterone

To study whether a low level of aldosterone might be responsible for a low percentage of ultrafiltrable potassium in the presence of a high percentage of ultrafiltrable sodium, which were frequently seen in cachectic patients, *dl*-aldosterone (Ciba A.G*) was added to four serums with this relationship of ultrafiltrable sodium and potassium.

Table 16. — *Effect of Aldosterone added to Serum in vitro on Ultrafiltrability of Sodium and Potassium*

Subject	Serum for Ultrafiltration	Serum		Ultrafiltrate	
		(Na) mEq./l. H_2O	(K) mEq./l. H_2O	(Na) mEq./l. H_2O	(K) mEq./l. H_2O
Pulmonary tuberculosis	O serum	146.0	4.70	140.0	4.26
	Control serum			139.0	4.20
	14 µg./ml. aldosterone added			138.5	4.17
„	O serum	144.0	4.70	141.0	3.91
	Control serum			140.0	3.96
	14 µg./ml. aldosterone added			138.0	3.93
Carcinoma of lung	O serum	135.0	5.22	125.5	4.47
	Control serum			127.5	4.50
	25 µg./ml. aldosterone added			129.0	4.52
Gamma myeloma	O serum	140.0	4.50	135.0	3.83
	Control serum			137.0	3.80
	25 µg./ml. aldosterone added			135.0	3.78

*) The author is indebted to Ciba A.G. for the generous gift of aldosterone.

Following the instructions of the manufacturer. 1 mg. of aldosterone was dissolved in 0.2 ml. of ethanol and a physiologic solution of sodium chloride and potassium chloride was added to make 2 ml. The resulting solution was added to the serum in a volume to give a concentration of 14—25 µg./ml. of added aldosterone in the serum. The same volume of ethanol-saline solution containing no aldosterone was added to the control serum. The serums were then kept at 38° C for 1 hour before ultrafiltration. The sodium and potassium contents of the ultrafiltrates are presented in table 16. It is observed that the sodium and potassium concentrations of the ultrafiltrates were practically equal in the case of all three serums, i.e., the original O serum to which nothing was added, the control serum to which had been added a small volume of ethanol-saline solution, and the aldosterone serum containing aldosterone-ethanol-saline solution.

Aldosterone added to serum *in vitro* was therefore not found to alter the ultrafiltrability of serum sodium and potassium.

Effect of Heparin

Experiments were made to compare the ultrafiltrability of sodium and potassium from heparinized plasma and serum. The blood samples were taken simultaneously and coagulation of the plasma samples was prevented by the addition of 0.05 ml. of a heparin preparation per 10 ml. of blood (Pularin, Orion Oy, containing 25,000 units of heparin per ml.). The blood from which the plasma samples were centrifuged thus contained 120 units of added heparin per ml. The sodium content

Table 17. — *Percentages of Ultrafiltrable Sodium and Potassium in Serum and in Heparinized Plasma*

Experiment No.	$\frac{100\ (Na)_f}{(Na)_s}\%$	$\frac{100\ (Na)_f}{(Na)_{plasma}}\%$	$\frac{100\ (K)_f}{(K)_s}\%$	$\frac{100\ (K)_f}{(K)_{plasma}}\%$
1	94.4	90.2	87.5	85.5
2	94.0	92.3	86.0	82.6
3	94.1	90.5	90.1	—
4	93.2	86.6	83.4	75.0
5	96.3	89.5	90.8	82.0
Mean	94.5	89.8	87.5	81.4

of the heparin was 1200 mEq./l., which raised the plasma sodium correspondingly. The heparin contained no potassium.

It is seen from table 17 that the mean percentage of ultrafiltrable sodium was 5.2 per cent higher and that of potassium 7.5 per cent higher in serum than in heparinized plasma. This clear decrease in the ultrafiltrability of plasma cations when compared to that of serum cations gave reason to presume that the heparin in the plasma was responsible for the difference. Wilander (1939), using the method of freezing point determination. observed a considerably lower molality of the calcium salt of heparin than when calculated from the calcium concentration of the solution. This points to the probability that heparin lower the osmotic activity of calcium.

To study the interactions of heparin with sodium and potassium. the ultrafiltrability of alkali metal ions was studied in solutions of sodium chloride and potassium chloride in water with added heparin. The following heparin preparations were used: Pularin (Orion Oy), Novo (Novo Industri), Medica (Ab Medica Oy) and Liquemin (Hoffmann-LaRoche & Co. A.G.). The preparations used in experiments 1 — 3 (table 18) contained 25,000 units of heparin per ml. and a tenfold dilution was made with an aqueous solution of sodium chloride and potassium chloride containing 145 mEq. of sodium and 4.5 mEq. of potassium per liter. In experiment 4 a preparation with a higher heparin concentration, containing 40,000 units per ml., was used. A fiftyfold dilution was made with a sodium chloride and potassium chloride solution containing 73 mEq. of sodium and 4.5 mEq. of potassium per liter. In experiment 5 was used a similar solution to which was added protamine solution (Protamin Sulfas 0.01 gm./ml. Orion Oy.) in the ratio 1:1. The undiluted heparin preparations used in experiments 1 — 3 contained *c.* 1200 mEq. of sodium per liter and those used in experiments 4 and 5 *c.* 2000 mEq./l. There were no appreciable amounts of potassium in any of the preparations.

It is observed in table 18 that heparin clearly reduces the ultrafiltrability of sodium and potassium. The percentage of ultrafiltrable potassium in experiments 1, 3 and 4 was still smaller than that of sodium although all the potassium in the heparin solution was added in the form of potassium chloride. This seems to indicate that heparin acts like an ion exchanger.

Table 18. — *Ultrafiltrability of Sodium and Potassium from Heparin Solutions Diluted with Aqueous Solutions of Sodium Chloride and Potassium Chloride*

Experiment No.	Heparin Preparation and Concentration	Heparin Solution			Ultrafiltrate		100 Na_f / $Na_{hepar.}$ %	100 K_f / K_{hepar} %
		pH	Na mEq./l.	K mEq./l.	Na mEq./l.	K mEq./l.		
	"Pularin" 2500 I.U./ml.	7.65	283	4.59	171	2.21	60.5	48.2
	"Novo" 2500 I.U./ml.	7.09	291	4.95	173	3.07	59.4	62.1
	"Medica" 2500 I.U./ml.	6.97	330	4.62	185	2.26	56.0	49.0
	"Liquemin" 1000 I.U./ml.	5.20	123	4.34	92	2.80	75.0	65.0
	"Liquemin" 500 I.U. + Protamine 0.005 gm./ml.	—	63.5	2.20	59.0	1.84	92.8	82.8

Using Donnan's theory it is possible to calculate the amount of cations that have been bound by heparin. According to this theory the products of ultrafiltrable ions in the solutions on the two sides of the membrane are equal (p. 9, equation 1). In experiments 1—3 the mean cation concentration on the ultrafiltrate side was 179 mEq./l., the ionic product thus being $179^2 = 32{,}000$. On the side of the heparin solution the mean chloride ion concentration was 150 mEq./l. In order that Donnan's theory would be fulfilled, cation concentration x on the heparin side should be $150\ x = 32{,}000$, of which $x = 214$ mEq./l. The concentration of sodium and potassium on the heparin side determined by flame photometry was, however, 306 mEq./l. Thus in experiments 1—3 the mean amount of cations bound by heparin was $306 - 214 = 92$ mEq./l.

The result can also be expressed in the probably more correct form that in the solutions having a heparin concentration of 2500 units per ml. the activity coefficient of sodium and potassium is $\frac{214}{306} = 0.7$ of the activity coefficient of sodium chloride and potassium chloride of the same concentration in an aqueous solution.

Certain other anionic polysaccharides, such as chondroitin sulfate (Farber *et al.* 1957) and arabic acid (Veis 1953), have been previously found to bind sodium and potassium. Some synthetic polyelectrolytes, such as polyacrylate also, bind alkali metals (Wall and Doremus 1954).

DISCUSSION

Ultrafiltrability of Serum Sodium and Potassium. — The results presented in tables 7—11 indicate that the relative amounts of ultrafiltrable serum sodium and potassium differ in normal and pathologic serums. These variations are correlated chiefly to changes in the serum albumin concentration. With a decrease of the albumin concentration by 1 gm. per 100 ml. the percentage of ultrafiltrable sodium increases by an average of 0.8 per cent and that of potassium by an average of 1.4 per cent (figs. 3 and 4). The percentages of ultrafiltrable sodium and potassium in 4 per cent and 5 per cent solutions of albumin and the potential difference between the albumin solution and the ultrafiltrate are, at the physiologic pH level, of the same order of magnitude as those of normal serum (table 6). This also is evidence indicating that albumin is the main factor in the respects discussed.

It may be possible that changes in the concentrations of the various globulin fractions also influence the ionic distribution, since, for example, the isoelectric points of the globulins differ slightly. On the basis of the present work it is difficult to evaluate the possible effect of the different globulin fractions since the effect of albumin appears to be dominant. The facts that sodium and potassium are completely ultrafiltrable from γ-globulin solution (table 6) and that there is no noteworthy potential difference between the γ-globulin solution and its ultrafiltrate suggest that the changes demonstrable in at least this globulin fraction have no influence on the ultrafiltrability of serum sodium and potassium and on the membrane potential.

It has been found that the percentage of ultrafiltrable calcium depends upon the serum proteins, chiefly albumin, as appears from the survey by Prasad (1960). The percentage of ultrafiltrable magnesium, likewise, depends mostly upon the albumin concentration

(Copeland and Sunderman 1952. Prasad *et al.* 1959). It appears, however, that no experiments have previously been carried out concerning the effect of changes in serum proteins seen in various diseases on the ultrafiltrability of sodium and potassium.

The differences in the ultrafiltrability of sodium observed in the various groups of this work correspond fairly well with the effect, according to Donnan's theory, of the differences in serum proteins in these groups. This becomes evident from the observation that the cation equivalency of serum proteins calculated by Van Slyke's formula from the concentrations of proteins and the pH levels corresponds in each group fairly well with the cation equivalency of serum colloids calculated according to Donnan's theory from the ultrafiltrability of sodium (table 13). The ultrafiltrability of potassium differs in certain respects from that of sodium. In all the groups the percentage of ultrafiltrable potassium was smaller than that of ultrafiltrable sodium; furthermore, the ratio: percentage of ultrafiltrable sodium/percentage of ultrafiltrable potassium varied in the different groups (table 12). In other words, the selective affinity of the serum colloids for these ions was variable.

Membrane Potential and Selective Distribution of Sodium and Potassium across a Membrane. — Already Liebig (1847) found that serum contains much sodium and but little potassium, whereas the relationship is the inverse in muscle fluid. The mechanism causing this is still poorly known. There are electrochemical differences between sodium and potassium ions in the respect, for example, that the conductivity of a potassium chloride solution is greater than that of a sodium chloride solution of the same concentration. Potassium migrates toward the cathode faster than sodium, suggesting that the hydration of the potassium ion is smaller than that of the sodium ion. Calculated from their conductivities, the hydrated radius of the potassium ion is 2.0 Å and that of sodium 2.6 Å (e.g., Harned and Owen 1950). It has been postulated by Ling (1952) that due to these differences a negatively charged protein network adsorbs potassium in preference to sodium. According to his hypothesis, the negative charge of the intracellular fluid in relation to the extracellular fluid (nearly —100 mV in the resting state) is due to a high intracellular concentration of organic anions, such as proteins, adenosine triphosphate, creatine phosphate and hexose monophos-

phate, and this would be the primary factor in the selective ionic distribution.

The mean potential difference between the serum and the ultrafiltrate in the control group in the present work was —9.3 mV, the serum being negative in respect to the ultrafiltrate. However, assuming that the sodium activity coefficients are equal in the serum and the ultrafiltrate, and calculating the membrane potential (equation 5) from the sodium concentrations of these fluids, the membrane potential in the control group would be — 2.0 mV. Similarly in the hepatic cirrhosis, nephrosis and pulmonary tuberculosis groups the measured membrane potentials ranged from —5.5 to —6.5 mV and the calculated potentials from —1.1 to —1.4 mV. In the renal insufficiency group the measured value was —7.2 mV and the calculated value —1.6 mV. The differences between the measured and the calculated membrane potentials are therefore fairly great. Uniformity exists between them, however, in the respect that in the control and in the renal insufficiency groups the membrane potentials determined in the two manners are greater than the values determined in the same way in the other groups. It seems difficult to find an explanation for the difference between the measured and the calculated membrane potentials. There are available some values for the potential between the serum and the equilibrium dialysis fluid. Lehman and Meesman (1924) obtained a membrane potential of from —6 to —8 mV between plasma and the dialysis fluid. Those between plasma and aqueous humor and between plasma cerebrospinal fluid were of the same order. These values are in agreement with the measured values obtained in the present study. Hecht (1925), on the other hand, obtained a membrane potential of from —1.8 to —2.3 mV between serum and the dialysis fluid, which conforms well with the theoretic value.

The observation in the present work that negatively charged serum adsorbs potassium in excess of sodium is in agreement with Ling's hypothesis. In examining the correlation between the quantities of membrane potential and selective adsorption of potassium in preference to sodium it was observed, however, that in this respect a correlation exists only in the renal insufficiency group, in which the adsorption of potassium in preference to sodium increased with increasing membrane potential (fig. 2). The literature contains an investigation by Tsao and Levinthal (1957) which may have some

bearing on this correlation. They found that after subtracting from the total cations determined by electrolysis the sum of sodium, potassium and calcium, the serum of a uremic patient may contain in excess as much as 40 mEq./l. of unidentified cations. They were unable to analyse the chemical nature of these apparently organic cations. In other diseases, for example fever, septicemia and liver diseases, as well as in healthy subjects they observed no excess of unidentified cations.

Ling's hypothesis concerning the significance of the membrane potential and the charge of organic anions in the selective distribution of body sodium and potassium was severely criticized by Conway (1957). On the other hand, Ussing (1960), for example, regarded it as a serious alternative hypothesis for the theories of active transport of alkali metal ions. According to the latter theories the relation between carbohydrate metabolism and potassium interchange, for example, can be explained by the active transport of potassium (Conway and Boyle 1939). Ussing (1960) has reviewed results suggesting the active transport of sodium by frog skin. Such transports would call for the existence of carriers which would form complexes specifically with sodium or potassium. In this connection it is interesting that many enzymes need for their activation a specific metallic ion, as is seen from the survey by Ussing (1960). For example, adenosinetriphosphatase is activated by sodium but not by potassium (Järnefelt 1961). However, using membrane electrodes of negatively charged collodion, Snell (1955) and Tosteson (1957) found that several biologically active phosphate anions have no selective affinity for sodium or potassium.

It has been demonstrated that calcium may form negatively charged small-molecular complexes, the formation of which enhances the ultrafiltrability of calcium (Manery 1954). Studies have also been published supplying evidence that a part of the serum potassium exists in a negatively charged form. Waelsch and Kittel (1934) reported that in the cataphoresis of normal serum the greater proportion of the potassium migrated to the anode. This portion was decreased in diabetics. Somogyi (1940) found that 81 per cent of the serum potassium of normal animals migrated to the cathode and 4 per cent to the anode, while the remainder was left in the serum. Following adrenalectomy 90 per cent of the potassium migrated to

the cathode and 2.6 per cent to the anode. However, it seems that these interesting experiments have not been repeated.

Wilbrandt (1959) postulated that the adrenal mineralocorticoids may act as carrier by forming chelate complexes with alkali metal ions. For this reason an attempt was made to determine whether the high percentage of ultrafiltrable sodium and the concomitant low percentage of ultrafiltrable potassium observed in the present work in cachectic patients would be due to a possible low serum aldosterone and whether the opposite quantities of these ultrafiltrabilities seen in the hepatic cirrhosis and nephrosis groups could be explained by a high serum aldosterone. The aldosterone added *in vitro* was found not to influence the ultrafiltrability of serum sodium or potassium, however. This finding suggests that the differences observed in the selective distribution of sodium and potassium across an artificial membrane cannot be ascribed to possible differences in the serum aldosterone. Recently reports have been published pointing to the probability that aldosterone is not a contributing factor in sudden changes in the sodium/potassium ratio *in vivo* (e.g., Ganong and Mulrow 1958, Ross *et al.* 1959).

It seems that nothing definite can be said as to whether the mechanism of selectivity for sodium and potassium observed in the present work is the effect of the membrane potential due to the serum organic anions according to Ling, or that of specific alkali metal carriers possibly present in the serum, or whether it is some other factor not discussed here. Further studies are in progress concerning the effect of the membrane potential on the selective distribution of sodium and potassium. The fact that differences in the selectivity between sodium and potassium were observed on ultrafiltration of serum across an artificial membrane indicates in any case that they are due to chemical or physicochemical differences in the serums and not to an "active" transport of ions by living protoplasm.

Interactions of Heparin with Sodium and Potassium. — Heparin is an example of a polyanion that binds sodium and potassium. The percentage of ultrafiltrable potassium in the heparin solution was in most cases lower than that of ultrafiltrable sodium, suggesting that heparin binds potassium in preference to sodium and thus acts like an ion exchanger. Similar results have been obtained in later experiments (Salminen and Luomanmäki, to be published). The effect of heparin on the ultrafiltrability of sodium and potassium cannot

be explained according to Donnan's theory if the calculations are based on the total concentrations of sodium and potassium in the heparin solution as determined by flame photometry, but it must be presumed that heparin lowers the activity coefficient of sodium and potassium (table 18). Hammarsten (1924) was probably the first to demonstrate by Donnan's theory that thymonucleic acid binds sodium. In the same manner Christensen (1940) showed that cephalin binds sodium and potassium. Further, Farber *et al.* (1957), applying Donnan's theory, found that chondroitin sulfate binds sodium and potassium and that the activity coefficient of potassium declines in most cases more than that of sodium. Using a membrane electrode of negatively charged collodion, Carr (1956) noticed that certain proteins lower the activity of sodium and potassium and that the serum colloids lower the activity of sodium by a barely measurable amount, whereas that of potassium is more clearly decreased.

Further literature and discussions on the interactions of sodium and potassium with organic molecules as well as on the influencing physicochemical factors have been quoted by Ussing (1960).

There are prospects that in the future it may be possible to make direct measurements of sodium and potassium activity with a glass membrane electrode in the same manner as it now is possible to measure the activity of the hydrogen ion with a membrane electrode made of glass sensitive to hydrogen ion. Eisenman *et al.* (1957) have succeeded in developing glass qualities from which membranes can be made with a potential related specifically to the activity of the sodium ion, for example. Experiments to measure the activities of sodium and potassium in biologic fluids have already been successful (Friedman *et al.* 1958, Hinke 1959).

Effect of pH Distribution of Sodium and Potassium. — Since the dissociation of proteins is dependent on the pH of the solution, it would be expected according to Donnan's theory that the ultrafiltrability of sodium and potassium would depend on the pH. Loeb (1928) has shown that the ultrafiltrability of calcium is influenced by the serum pH which has been changed *in vitro*. Ingraham *et al.* (1933) found that the ultrafiltrability of sodium and potassium increased when the serum pH was lowered by carbon dioxide. In similarity to the above findings, table 6 shows that large changes in pH have a definite effect on the ultrafiltrability of sodium and potassium

from an albumin solution. Changes in pH occurring in the body, however, are considerably smaller than those effected *in vitro* in the above mentioned experiments. In the present series the serum pH level of the most acidotic patients (renal insufficiency) was 7.10, and that of the most alkalotic patients (hepatic cirrhosis) was 7.50. Assuming that serum contains 4 mg. of albumin and 3 mg. of globulin per 100 ml., the cation equivalency of serum proteins in such a serum is according to Van Slyke (equation 7) 15 mEq./l. at pH 7.10 and 18 mEq./l. at pH 7.50. According to Donnan's theory (equation 2), this would make the sodium concentration of the ultrafiltrate of the most acidotic serum slightly over 1 per cent greater than that of the ultrafiltrate of the most alkaline serum. This difference would be just visible with the flame photometer used. Since in the present series the protein concentrations of the individual serums vary greatly, a possible pH effect is overshadowed by the changes produced in the ultrafiltrability of sodium and potassium by variations in protein concentration. This series therefore does not justify conclusions concerning the effect of clinical alkalosis or acidosis on the ultrafiltrability of serum sodium and potassium.

Although the effect of pH on the distribution of the serum ions is small according to theoretic calculations, it may have an effect on the ionic distribution in the body, since the cation equivalency of intracellular proteins is considerably greater than that of serum. An indication of the effect of the serum pH on the ionic distribution is the observation made by many investigators that with a fall in the pH of serum or of extracellular fluid in general the potassium is transferred from the intracellular to the extracellular space, resulting in an increase in the potassium concentration of the extracellular fluid (e.g., Fenn and Gobb 1934, Abrams *et al.* 1951, Cotlove *et al.* 1951, Scribner *et al.* 1955, Burnell *et al.* 1956, Schwartz *et al.* 1957, Fenn *et al.* 1958, Simmons and Avedon 1959). The last mentioned workers have collected the results of studies made up to 1959, which show that with a mean fall of the serum or plasma pH by 0.1 unit the potassium concentration rises by 0.46 mEq./l. In the above mentioned investigations the changes occurring in a patient's or experimental animal's extracellular fluid were determined during an acute experiment. Leibman and Edelman (1959), however, reported that a similar correlation can be demonstrated even when each serum sample is taken from a different individual in a greatly

heterogeneous series of patients, and they observed that the correlation is enhanced when the plasma potassium concentration is plotted against the product of the plasma hydrogen ion concentration and the ratio: total exhangeable potassium per kg. dry body weight/plasma sodium concentration. It may be calculated from their results that an increase of *c.* 1 mEq./l. in the serum potassium corresponds to a fall of 0.1 pH unit. A similar correlation is observed in the present work, in which the series studied was collected in the same manner as in the investigation of Leibman and Edelman. In this work a drop of 0.1 pH unit is equivalent to an increase of 0.75 mEq./l. in the serum potassium (cf. fig. 8). The greatest deviation from the regression line is seen in the case of certain patients with renal insufficiency. For example, patients No. 84, 88 and 89 had a lower potassium concentration than would have been expected on the basis of the pH level. Labhardt and Spühler (1953) also observed that hypokalemia may be seen in renal insufficiency regardless of whether alkalosis or acidosis is present.

The mechanism responsible for the interrelationship between the pH and the potassium concentration of extracellular fluid can be explained, with some exceptions, on the basis of Donnan equilibrium existing between intracellular and extracellular fluid (Fenn *et al.* 1958).

The activity of the hydrogen ion can be directly measured by available methods. Thus the membrane potential between two solutions can be very well calculated from the hydrogen ion activities of the solutions. The formula for the membrane potential at 38° C (equation 5) then changes to the following simple form:

$$E_{mV} = 62\ (pH_I - pH_{II})$$

Loeb (1921) has shown that the membrane potential between a gelatin solution and its dialysis fluid calculated by this formula agrees well with the membrane potential measured with calomel electrodes using saturated KCl bridges. The pH of the ultrafiltrate was not measured in the present work since the new type of microelectrode for anaerobic measurement of pH at 38° C (Astrup 1959) was not yet available, and formation of an adequately large volume of ultrafiltrate for measurement by the older type of electrode would not have been possible with the ultrafiltration system used. However, the pH of some ascites fluid samples was measured, making it possible

to compare the membrane potential calculated from the pH of the serum and ascites fluid with the measured membrane potential between serum and ultrafiltrate. Using serums of the same patients, the first mentioned mean membrane potential (cf. table 15) was —4.6 mV, while the latter was —3.1 mV. These values are of the same order. This is in agreement with the finding by Hastings *et al.* (1926) that the hydrogen ion is distributed in the body between the serum and ascites fluid in the same manner as it is ultrafiltrated from the serum across a collodion membrane.

Hyponatremia in the Diseases Studies. — Hyponatremia is known to be a common finding in patients who are in a poor condition from the most varied causes, as appears, for example, from the survey by Edelman (1956). In the present study, also, the serum sodium in all the disease groups was lower than in the control group (cf. tables 7—11).

The lowest sodium concentration was seen in the pulmonary tuberculosis group. Sims *et al.* (1950) also observed that patients with pulmonary tuberculosis frequently are hyponatremic without, however, exhibiting symptoms of hyponatrèmia. In the present study the mean serum sodium concentration of patients with pulmonary tuburculosis was *c.* 9 mEq./l. lower than that of the controls, while the sodium concentration of the ultrafiltrate was only *c.* 5 mEq./l. below the controls. In the hepatic cirrhosis and nephrosis groups, also, the sodium concentrations of the ultrafiltrates did not differ from the controls as much as did the serum sodium concentrations. The reason for this is the greater percentage of ultrafiltrable sodium in the disease groups as compared with the control group (cf. tables 7—11). The biologically significant sodium concentration, which probably is better represented by the ultrafiltrate sodium than by the serum total sodium, is therefore not as greatly reduced in hyponatremic patients as might be concluded from the serum total sodium concentration. A similar tendency is observed in the case of potassium in the respect that hypokalemic patients frequently have an elevated percentage of ultrafiltrable serum potassium (fig. 7), even though the correlation is not as clear as in the case of sodium. In analogy, hypocalcemia is often accompanied by a high percentage of ultrafiltrable serum calcium and such patients frequently show no symptoms of hypocalcemia (e.g., Terepka *et al.* 1958).

It is to be noted, however, that the ultrafiltrate sodium concentrations in the pathological series, with the exception of the nephrosis, did not attain the level of the ultrafiltrate sodium concentration in the control group. Therefore it seems that the increased ultrafiltrability of serum sodium in hyponatremic patients surely does not provide a full explanation for the hyponatremia seen in the diseases in question.

The serum sodium level does not provide an indication of the sodium concentration in other body fluids. The finding that the sodium concentration in the edema fluid of patients with toxemia of pregnancy was greater than in non-toxemic gravidas although the serum sodium concentrations were the inverse (Tatum 1954) is evidence that the sodium concentration of even interstitial fluid need not be directly related to the serum sodium concentration. The sodium concentration of the intracellular fluid is frequently increased in hyponatremic patients (e.g., Jahrmärker 1959). In edemic patients the serum sodium correlates poorly with the total exchangeable body sodium concentration per liter of body water. Thus this concentration is increased in cirrhosis of the liver (Farber and Soberman 1956, Talso *et al.* 1956, Birkenfeld *et al.* 1958). Even in hyponatremic patients with this disease the concentration of total exchangeable sodium per liter of body water is increased, and therefore simple overhydration cannot provide an explanation for the hyponatremia (Talso *et al.* 1956).

The osmolality of body fluids depends to a great extent on the concentration of sodium or potassium. Results obtained by the freezing point method show that the osmolalities of the extra- and intracellular fluids are equal (e.g., Conway and McCormack 1953, Maffly and Leaf 1958). In agreement with these facts is the observation that the serum sodium concentration of a heterogeneous series of patients with, among others, heart diseases, cirrhosis of the liver, pulmonary tuberculosis and kidney diseases correlates well with the sum of the total exchangeable sodium and total exchangeable potassium per liter of body water (Edelman *et al.* 1958). A further finding suggesting that potassium influences the distribution of sodium is that the serum sodium in hyponatremic patients increases on administration of potassium (Laragh 1954). The low plasma sodium is often due to loss of potassium in excess of water (Wynn 1957). The potassium in the intracellular fluid is opposed by the

organic anions (proteins, etc.), the concentration of which is markedly greater in this fluid than in serum. Edelman (1956) has advanced a theory that a decreased concentration of intracellular anions may be one of the primary reasons for the lower osmolality of body fluids and therefore also for a low serum sodium concentration.

Our knowledge of the chemistry of the anionic pattern of intracellular fluid is deficient (Weidmann 1957). It may be fitting to call organic anions the "battery" of biological systems (Briller 1959). Might a decrease in the amount of these organic anions provide an explanation according to Ling's hypothesis for the lowering of the selectivity between body sodium and potassium which occurs in the hypo-osmolality syndrome?

Owing to methodic difficulties, the contribution of intracellular anions in the regulation of the osmolality of body fluids has only been demonstrated so far indirectly on basis of the behavior of potassium. However, the interactions between the serum colloids and small ions can be studied more easily. Thus Broch (1945) observed that the "base binding power of serum proteins" (which in the present work is termed the "cation equivalency of serum colloids") has a correlation with the concentration of total cations in the serum. With a fall in the "base binding power of serum proteins" there is a decrease in the serum total cation concentration because of a reduction in the Donnan effect that retains cations in the serum (Broch 1945). The flame photometer was not yet widely used at the time of Broch's studies and a determination of the sodium concentration was therefore not made, but since the serum cations consist mostly of sodium, Broch (1953) assumed that the "base binding power of serum proteins" is correlated also to the serum sodium concentration in the same manner as to the total cation concentration. The results obtained in the present investigation support this opinion. The serum sodium concentrations decreased with increasing percentages of ultrafiltrable serum sodium ($r=-0.36$, $P < 0.001$) and with decreasing cation equivalencies of serum colloids ($r=0.59$, $P < 0.001$).

Although this heterogeneous series of patients revealed a significant correlation between the serum sodium concentration and the cation equivalency of serum colloids, it does not appear probable that serum colloids would be the only or primary factor capable of infuencing the sodium concentration of serum and of all fluids which are in equilibrium with the serum. Some investigators have reported

that serum proteins are in equilibrium with tissue proteins, as is evident from the review by Wuhrman and Wunderly (1947). This possible equilibrium might become the connecting link between the theory of Edelman (1956) and the observations in the present work concerning the regulation of the serum sodium concentration.

Other results have also been published pointing to the influence of proteins and especially of albumin on the sodium distribution. It can be seen from results of Luetscher *et al.* (1950) and Ricketts *et al.* (1951). that the serum sodium concentration of patients with hypoalbuminemia and hyponatremia increases after an infusion of salt-poor concentrated albumin. According to Greese *et al.* (1958) the accumulation of sodium from physiologic saline solution into the cells of a tissue slice is inhibited when serum proteins are added to the saline solution.

SUMMARY

The ultrafiltrability of sodium and potassium in serum and in certain protein solutions was studied in this investigation. A further subject of investigation was the effect of aldosterone and heparin added *in vitro* on the state of sodium and potassium. The work was carried out by comparing the sodium and potassium concentrations in the water of ultrafiltrate obtained by forcing colloid fluid through a dialyzing membrane to the corresponding ionic concentrations in the water of the colloid fluid. Determinations were also made of the potential difference between the colloid fluid and its ultrafiltrate, the pH of the colloid fluid, and the total concentration and electrophoretic fractionation of the serum proteins.

Experiments with the protein solutions showed that the percentage of ultrafiltrable sodium in 4 per cent and 5 per cent solutions of albumin was *c.* 93 per cent and that of ultrafiltrable potassium *c.* 90 per cent at the physiologic pH. The potential of the protein solution was then negative in relation to the ultrafiltrate. With decreasing concentrations of albumin the ultrafiltrability of the cations increased and membrane potential difference decreased. When the pH of the albumin solution was changed to the acid side of the isoelectric point of albumin, the sodium and potassium concentrations of the ultrafiltrate were higher than the corresponding cation concentrations of the albumin solution and the membrane potential on the albumin side was positive. These changes are in agreement with Donnan's theory. The sodium and potassium were completely ultrafiltrable from the γ-globulin solution at physiologic pH and no potential difference was seen between the γ-globulin solution and its ultrafiltrate.

A total of 94 serum samples were examined. They were obtained from the following test subjects: 27 healthy persons, 24 patients

with cirrhosis of the liver, 6 patients with nephrosis, 16 patients with pulmonary tuberculosis, and 18 patients with renal insufficiency.

The mean percentage of ultrafiltrable serum sodium in the healthy subjects was 92.9 ± 0.27 per cent. In all the disease series it was higher than in the control group, as follows: Hepatic cirrhosis, 95.2 ± 0.33 per cent; nephrosis, 96.2 ± 0.73 per cent; pulmonary tuberculosis, 96.1 ± 0.42 per cent; and renal insufficiency, 94.5 ± 0.44 per cent.

The mean percentages of ultrafiltrable serum potassium were as follows: Controls, 88.6 ± 0.37 per cent; hepatic cirrhosis, 92.3 ± 0.56 per cent; nephrosis, 94.1 ± 0.69 per cent; pulmonary tuberculosis, 89.1 ± 0.78 per cent; and renal insufficiency, 90.4 ± 0.71 per cent. In the hepatic cirrhosis, nephrosis and renal insufficiency groups the mean percentage of ultrafiltrable potassium was higher than in the control group. In the pulmonary tuberculosis group it did not differ significantly from the control group.

The selective adsorption of potassium in preference to sodium by serum colloids (ratio: percentage of ultrafiltrable serum sodium/ percentage of ultrafiltrable serum potassium) was lowest in hepatic cirrhosis and nephrosis and highest in pulmonary tuberculosis. The control and renal insufficiency groups were intermediary in this respect.

It was found in renal insufficiency, that with increasing potential difference between serum and ultrafiltrate there was an increase in the serum adsorption of potassium in preference to sodium.

The mean membrane potential between serum and ultrafiltrate was —9.3 ± 0.46 mV in the control group, the serum being negative in relation to the ultrafiltrate. The membrane potential difference was lower in all the disease series, as follows: Hepatic cirrhosis, —6.0 ± 0.72 mV; nephrosis, —6.5 ± 0.98 mV; pulmonary tuberculosis, —5.5 ± 0.5 mV; and renal insufficiency, —7.2 ± 0.87 mV.

The mean serum pH in the control group was 7.385 ± 0.0044. In the renal insufficiency group it was 7.273 ± 0.021 and in the pulmonary tuberculosis group 7.350 ± 0.018, which were lower than in the control group. The pH 7.346 ± 0.031 in the nephrosis group did not differ significantly from the controls. It was above the control group level in the hepatic cirrhosis group, in which it

was 7.420 ± 0.011. The pCO_2 determined in 5 patients with hepatic cirrhosis was low, suggesting that the alkalosis in their case was respiratory in origin.

The following correlations were noted, based on the results in all the cases:

— The percentages of ultrafiltrable serum sodium increased with decreasing serum albumin concentrations;

— The percentages of ultrafiltrable serum potassium increased with decreasing serum albumin concentrations;

— The percentages of ultrafiltrable serum sodium increased with decreasing concentrations of this cation in the serum;

— With decreasing cation equivalency of serum colloids the serum sodium concentration declined. This correlation was more evident than the correlation of the percentage of ultrafiltrable serum sodium to the sodium concentration of the serum;

— With a rising serum pH the serum potassium concentration decreased.

When the composition of the ascites fluid of 5 patients with hepatic cirrhosis was compared with the composition of the serum ultrafiltrate of the same patients, practically the same sodium concentrations were found in the two fluids, whereas the potassium concentrations in the fluids varied in different direction. The ascites fluid was, on the average, 0.07 pH unit more alkaline than the serum. The membrane potential between the ascites fluid and the serum calculated from their pH values was —4.6 mV. The measured membrane potential between the same serums and their ultrafiltrates was —3.1 mV.

The addition of aldosterone to the serum *in vitro* was found not to influence the ultrafiltrability of sodium or potassium.

In heparinized plasma the percentage of ultrafiltrable sodium was 5.2 per cent lower and that of potassium 7.5 per cent lower than the percentages in the serum.

Using the Donnan theory, it was found that heparin lowers the activity coefficient of alkali metal ions in an aqueous solution of heparin, sodium chloride and potassium chloride.

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